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A ROT OF WINTER QUEEN WATERMELONS CAUSED BY PHYTOPHTHORA CAPSICI[1]

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INTRODUCTION

Winter Queen watermelons,[2] known also as winter watermelons, are found on the New York market during September and October. In limited quantities they meet the demand for a late-season watermelon, while many of smaller size are pickled whole and eaten as a delicacy by certain groups.

The annual unloads of Winter Queen watermelons on the New York market, from 1928, the first year that separate records were kept, to 1938, inclusive, were as follows:[3]

Year:	*Carloads*
1928	13
1929	11
1930	28
1931	36
1932	29
1933	27
1934	14
1935	16
1936	7
1937	3
1938	4

It will be seen that unloads increased from 13 carloads in 1928 to a peak of 36 in 1931 but thereafter decreased markedly so that by 1937 only 3 carloads were handled, and in 1938, 4 carloads. Although few in number during 1937 and 1938, the melons arrived in good condition, were well received by the trade, and brought encouraging returns on the market. It appears reasonable to expect that annual unloads will increase substantially over the low point reached in 1937 and 1938.

In September 1935 a carload of Winter Queens arrived at New York City with approximately 80 percent of the melons affected with a phytophthora rot. Although a field and local market decay of Iowa Belle watermelons, caused by a species of *Phytophthora*, had been reported from Arizona (*2*),[4] this was the first record of a phytophthora rot of Winter Queen melons. The seriousness of the decay when

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[2] The Winter Queen is a variety of watermelon (*Citrullus vulgaris* Schrad.) that is thought to have been introduced into this country by immigrants from the Volga region of the Union of Soviet Socialist Republics. The fruits are small, nearly round, approximately 9 to 9½ inches in diameter, and weigh about 10 to 15 pounds. Although a few melons of large size (11 to 12 inches in diameter) are packed for the market, most are of the smaller sizes, many being about 6 inches in diameter. The rind is greenish to light cream in color with faint irregular stripes of light green. The pulp is bright red, stringless, of fine texture, firm, and sweet. The seeds are small and black. The melon is grown in the cantaloup- and Honey Dew melon-producing section of Colorado, packed in flat crates similar to those used for Honey-Dew melons, and shipped to the market in refrigerator cars without ice, either under standard ventilation or with vents open, to destination.

[3] Data taken from the annual reports of the unloads of fruits and vegetables at New York City, issued in mimeographed form by the Bureau of Agricultural Economics, U. S. Department of Agriculture.

[4] Italic numbers in parentheses refer to Literature Cited, p. 88.

first observed on the market suggested the advisability of learning more about its occurrence and economic importance. Periodic examinations were therefore made of these melons on the unloading and sales pier at New York during the remainder of the 1935 season as well as during the following three seasons. A study was likewise made of the identity and pathogenicity of the species of *Phytophthora* involved, the manner of infection of the melons, the spread of the disease from melon to melon within the container, and the temperature relations of the pathogen and of the disease. The present paper summarizes the results of these studies, made over a period of four marketing seasons, and makes available such information as has been obtained regarding the control of the disease.

REVIEW OF LITERATURE

Sherbakoff (*13*) in 1917 produced a decay of watermelon fruits by artificial inoculation with *Phytophthora parasitica* Dastur.[5]

A decay of watermelon fruits caused by *Phytophthora citrophthora* (E. H. and R. E. Smith) Leon., was reported from California (*4*) in 1929. Squash and pumpkin fruits were likewise found infected.

Brown and Evans (*2*) in 1933 described a fruit rot of Iowa Belle watermelons that was responsible for the loss of 40 to 50 percent of the crop in a 10-acre field near Marana, Ariz. The pathogen, *Phytophthora cactorum* (Cohn and Leb.) Schroet., was found capable, through artificial inoculation, of causing a similar decay of cucumber fruits.

Ramsey, Wiant, and Link (*10, p. 62*), in 1938 made brief mention of the decay of Winter Queen watermelons described in the present paper.

Reports of phytophthora rots of other cucurbit fruits include the following:

Curzi (*5*) produced a decay of the fruits of squash and those of *Bryonia dioica* Jacq. by artificial inoculation with *Phytophthora capsici* Leon.[6]

Drechsler (*6*) isolated a species of *Phytophthora* with prominently papillate sporangia from a Honey Dew melon found on the Washington, D. C., market after shipment from either California or Colorado. He produced a similar decay by artificial inoculation with *P. cactorum*, *P. citrophthora*, and *P. parasitica*.

Tompkins et al. (*14*) successfully inoculated watermelon and citron fruits with *Phytophthora drechsleri* Tucker from sugar-beet roots. The fungus was found capable of infecting fruits of summer squash through the unbroken skin as well as through wounds.

Tompkins and Tucker (*15*) described a field decay of Honey Dew melons in California, caused by *Phytophthora capsici*. The disease was transmitted by artificial inoculation to fruits of cucumber, pumpkin, squash, Klondike watermelons, and a number of types of muskmelon including cantaloups and Casaba, Honey Ball, Honey Dew, and Persian melons. *P. drechsleri* was involved to a slight extent. These authors (*16*) also found that fruits of pumpkin, watermelon,

[5] Described by Sherbakoff as a new species, under the name *P. terrestria*, (corrected to *terrestris*) but considered by Butler (*3*) and other students of the genus to be identical with *P. parasitica*.

[6] Described by Curzi as a new species, *P. hydrophila*, but considered by Tucker (*17*) to be identical with *P. capsici*.

and cucumber could be successfully inoculated through the unbroken skin with *P. cryptogea* Pethybr. and Laff. from China-aster.

Wiant (*18*) reported studies made on the New York City market of a phytophthora rot of Honey Dew melons from California, Colorado, and Chile. A similar decay of cantaloups and Honey Ball melons was produced by artificial inoculation.

Kreutzer (*7*) described a field rot of cucumber fruits causing a nearly complete loss of the crop in an 8-acre field located near Rocky Ford, Colo. He stated that the pathogen was probably *Phytophthora capsici*. Fruits of the squash were successfully inoculated with the fungus.

THE DISEASE

OCCURRENCE AND IMPORTANCE

Phytophthora rot of Winter Queen watermelons was first observed in a carlot unloaded at New York in early September 1935. An average of approximately 80 percent of the melons in the car were partly decayed, and in many of the crates all were infected. The decayed spots were mostly over 3 inches in diameter, with from one to three lesions per melon. The entire carlot was rejected for sale and was disposed of as garbage.

This car was shipped from Rocky Ford, Colo., 5 days previous to the date of unloading at New York. On the same day two other cars were shipped from Manzanola, Colo. These were unloaded at New York 5 and 7 days, respectively, after the date of loading. In the first of these from 15 to 50 percent of the melons were affected with phytophthora rot, with an average of 25 percent for the carlot. Approximately 15 percent of the melons in the second of these two carlots likewise were found to be in early to advanced stages of decay with phytophthora rot, while in a group of 73 crates nearly 40 percent of the melons were so affected.

Although observations were made on the condition of Winter Queen watermelons at the unloading and sales pier from time to time during the remainder of the 1935 season, only in the three carlots noted above was phytophthora rot found present. In this connection it is of interest to note that certificates issued by the Bureau of Agricultural Economics, United States Department of Agriculture, on eight additional carlots and two mixed carlots containing some Winter Queens indicated that four carlots (including one mixed carlot) were free of decay, while in six carlots (including one mixed carlot) from 1 to 8 percent (averaging 5 percent) of the melons were decayed. The decay was reported as alternaria rot and as an early stage of rhizopus soft rot. No inspections were made of the remaining three or four carlot equivalents of that season.

The unloading dates of the seven cars arriving at New York during the season of 1936 were scattered through a 5-week period; consequently, observations were limited. In one car, however, phytophthora rot was found, but the percentage of melons so affected was not determined. An examination of inspection reports on seven carlots (including one mixed lot), made either by the Bureau of Agricultural Economics or by the Railroad Perishable Inspection Agency, indicated 15 percent of decay in one carlot and from 1 to 5 (averaging 3.5) percent of decay in the other six carlots (including

one mixed lot). As during the previous season, the decay was for the most part recorded as alternaria rot and rhizopus soft rot.

No phytophthora rot was found in the seven carlots unloaded during 1937 and 1938. In fact, practically no decay of any kind was observed.

The results of the observations made over the four-season period may be summarized by stating that, out of a total of 30 carlots, phytophthora rot was definitely known to have occurred in 4, where it was responsible for from 5 percent or less to nearly a total loss of the melons. It definitely was not present in the 7 carlots of 1937 and 1938. Of the remaining 19 carlots, inspections by others on 15 to 16 carlots (or carlot equivalents, because of the mixed loads) suggest that, if phytophthora rot occurred at all, it was not responsible for heavy losses. However, this does not preclude the possibility that the decay may have been present in small amounts in some of the carlots without being recognized. It would appear that under certain conditions phytophthora rot may constitute a problem of the greatest importance in carlots of Winter Queen watermelons shipped to eastern markets.

SYMPTOMS

The earliest symptom of phytophthora rot is the development of either a slight water soaking or a reddish-brown ("vinaceous-tawny")[7] discoloration of the rind about the point of inoculation. When the water-soaking type of discoloration prevails, the lesion as it advances may become "grape green," "deep grape green," "deep olive-buff," "dark olive-buff," or "citrine drab" in color. Frequently there is little evidence of water soaking during the early stages of decay. Then the affected rind assumes colors of brown ("russet," "sayal brown," "snuff brown," "Saccardo's umber," or "mars brown").

The lesions are at first irregular in pattern (fig. 1, *A*). By the time they have attained a diameter of approximately 1 inch they become nearly circular in shape (fig. 1, *B*), although the margin may still be irregular. There may be one or several (fig. 1, *C*) lesions on a melon. When more than one occurs, adjacent lesions may coalesce in part as they enlarge. At the same time, isolated lesions assume a more nearly circular shape and their margins become regular and distinct (fig. 2, *A*). Frequently the lesions have a mottled appearance (fig. 2, *A*), or alternating concentric bands of several colors may be present (fig. 1, *C*).

In advanced stages of decay the white mycelium of the causal fungus develops on the affected areas of the melon (figs. 2, *A* and *B;* 3, *A* and *B*). This is first evident at the center of the lesion (fig. 2, *A*) and finally appears over all of the surface except that of the advancing edge of the lesion (figs. 2, *B;* 3, *A*). Such melons, confined within a closed moist chamber, develop a luxuriant white cottony mold growth. Under the conditions usually prevailing on the market, however, the mold does not grow out much from the surface but tends rather to become matted down against the moist tissues of the lesion.

The affected tissues early become somewhat softer than normal but retain their form for some time. Eventually there is shriveling and wrinkling with formation of creases in the diseased rind (figs. 2, *A;*

[7] All color designations in quotation marks in this paper are according to Ridgway (*11*).

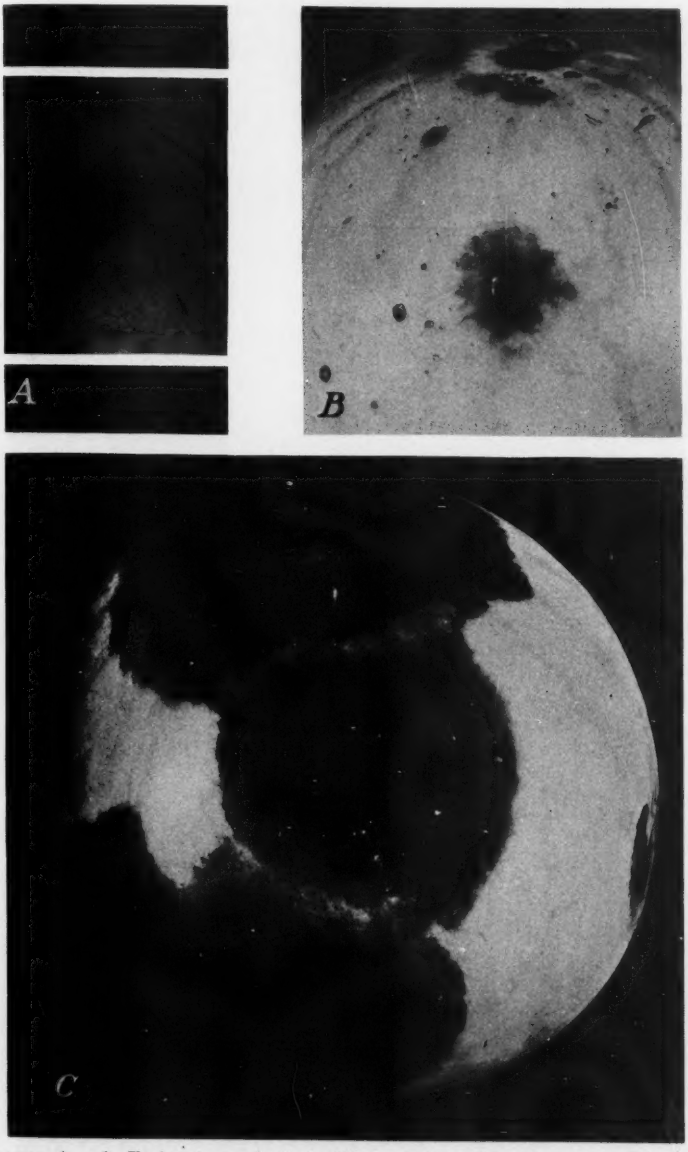

FIGURE 1.—*A*, Early stage of phytophthora rot resulting from inoculation of Winter Queen watermelon; *B*, more advanced stage; *C*, typical lesions on melons from commercial carlot.

FIGURE 2.—Phytophthora rot of Winter Queen watermelon: *A*, Lesion showing mottling, wrinkling, and beginning of mold development; *B*, older lesion nearly covered with mold.

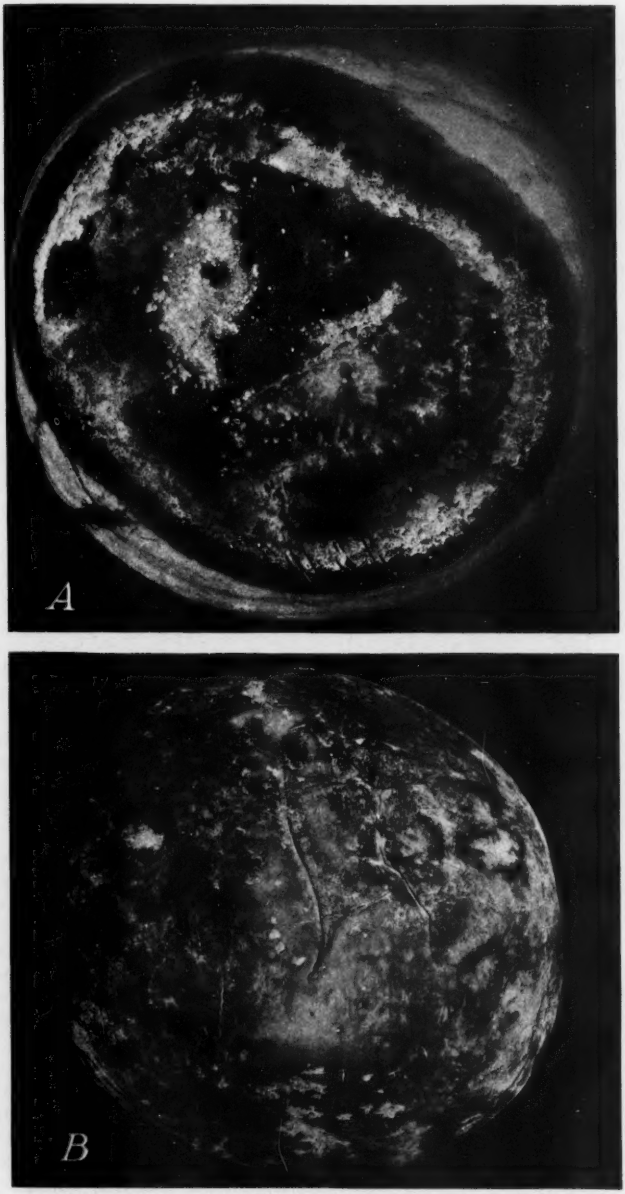

FIGURE 3.—Phytophthora rot of Winter Queen watermelon: *A*, Extensive lesion covering nearly half of melon surface; *B*, late stage of decay, entire melon showing shriveling, wrinkling, water soaking, and presence of wet appressed mold.

3, *B*), and the melon either flattens out under the pressure of its own weight (fig. 2, *B*) or otherwise loses its smooth, even, and regular shape (fig. 3, *B*). The epidermis becomes loosened soon after the rind is infected, and can be peeled off readily.

The internal symptoms of decay are a general shriveling and mashing together of the diseased tissues, which remain loosely held together by the fungus mycelium. When the moisture is squeezed out of bits of the affected tissue, the tough, felty mycelium remains as a slight residue. The flavor of healthy tissue somewhat beyond the decayed tissues, unless affected by contaminating yeasts or bacteria, is little affected by the decay. Likewise there occurs no marked odor or discoloration.

THE CAUSAL ORGANISM

MORPHOLOGY AND GROWTH CHARACTERS

Six isolates from rotting Winter Queen watermelon fruits were used for a study of the morphology and growth characters of the fungus. On potato-dextrose agar plates the isolates produced a dense white mat of mycelial growth in the agar, covered with a velvety growth of short aerial hyphae. There was no spore development during a 10-day period. In oatmeal agar tubes there was a mass of white, cottony, aerial mycelium at the base of the slope after 5 days' growth at room temperature. In pea broth, dense tufted masses of mycelium developed throughout the liquid in 6 days, but there was no spore development during that time.

Sporangia developed abundantly in oatmeal and cornmeal agar cultures kept at room temperature for 2 months; they were usually sphaerolimoniform, nonpedicellate, prominently papillate, with granular, hyaline to faintly straw-colored contents, 21.4μ to 53.1μ by 18.3μ to 40.7μ, averaging 36.5μ by 27.6μ in size (fig. 4, *A*). On solid media the sporangia were fairly uniform in size and shape. When the isolates were grown 6 days in pea broth there was a profuse development of mycelium; tufts of hypae washed and transferred to plates of sterile distilled water, as recommended by Leonian (*9*), developed sporangia in large numbers. They varied widely in size and shape, with a tendency to become elongated in the apical region; the majority had a single papilla, but irregular sporangia with two or three papillae were fairly common. The sporangia produced in water varied from 21.7μ to 60.4μ by 16.9μ to 43.8μ, averaging 40.6μ by 28.3μ in size. Germination of sporangia from both solid and liquid media occurred by the development of germ tubes, usually from the region of the papilla, and by the development and liberation of zoospores, which, after a period of motility, became quiescent, rounded up, and germinated by a single germ tube.

Chlamydospores were not observed.

Three of the six isolates developed oogonia and oospores in oatmeal agar cultures 2 months old. Isolates that developed sexual organs abundantly produced a few sporangia in the same cultures; however, the mycelium of the oogonium-producing isolates developed sporangia as abundantly as the non-oogonium-producing isolates when transferred from pea broth to sterile distilled water but oogonium development did not occur in the distilled water.

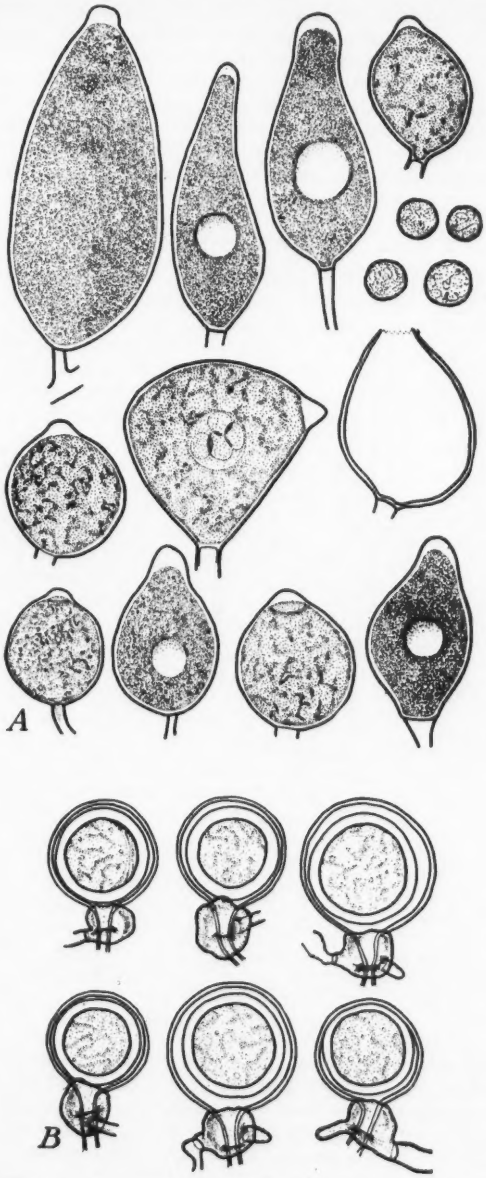

FIGURE 4.—*Phytophthora capsici* from Winter Queen watermelon: *A*, Sporangia, evacuated sporangium, and rounded, quiescent zoospores; *B*, oogonia, oospores, and antheridia. × about 600.

The oogonium was subspherical with a slender stalk encompassed by the persistent, amphigynous antheridium. The colorless oogonium wall enclosed dilutely straw-colored granular and often vacuolate contents, which, following fertilization and the differentiation of the oospore, became somewhat darker. The oospores were thick-walled, nearly spherical, not filling the oogonium; a single oospore developed in each oogonium. Oogonia measured 24.1μ to 41.4μ in diameter, averaging 29.9μ. The oospores measured 22.7μ to 31.4μ, with an average diameter of 26.5μ (fig. 4, *B*).

TAXONOMY

The causal organism was identified as *Phytophthora capsici* Leon. (*8*). The Winter Queen watermelon isolates were quite similar to strains of that species previously studied by Tucker (*17*).

Sarejanni (*12*) in 1936 proposed to combine *Phytophthora capsici* with *P. parasitica*. His proposal would place in the latter species all forms that grow well on malt extract agar and the usual agar media in 6 days at 20° C. and develop amphigynous antheridia and papillate sporangia. He suggested a new species, *P. imperfecta*, for strains with similar growth characters that do not develop oogonia. Strains differing in pathogenicity would be given varietal rank. According to his classification, the three present watermelon isolates that produced oogonia would be assigned to *P. parasitica* var. *capsici* and the three that did not develop oogonia to *P. imperfecta* var. *capsici*.

Conditions influencing the development of sexual organs in cultures of *Phytophthora* species are not well known. Their development is certainly affected by the age of the culture, temperature, substratum, moisture, and the occurrence of heterothallic strains, as well as other undetermined factors. Isolates that produce sexual organs abundantly when first cultured sometimes cease to produce them in later transfers, whereas those failing to produce them in early transfers occasionally develop oogonia and antheridia abundantly in later transfers. The character seems too variable for use as the sole basis of distinction between species of the group with amphigynous antheridia. Several species with nonpapillate sporangia develop oogonia infrequently in culture, yet possess other characters that permit their classification.

Phytophthora capsici is a fairly well defined species, distinguishable from *P. parasitica* by the consistent absence of chlamydospores in culture and by its pathogenicity to pepper plants, a character that has proved stable among isolates from various hosts. The retention of *P. capsici* as a species is therefore highly advisable.

TEMPERATURE RELATIONS

The temperature relations of *Phytophthora capsici* from Winter Queen watermelons were determined by measuring the growth that occurred on plates of potato-dextrose agar [8] during a period of 4 days at temperatures of 45° to 100° F., and during 1 week and 2 weeks at 45°, 50°, and 55°. The results are summarized in figure 5, *A*. The data there shown for the temperatures within the range of 55° to 90°, inclusive, are averages of 4 experiments in each of which 10 plates were measured at each temperature. Thus each point on that

[8] Pared potatoes, 200 gm.; dextrose, 20 gm.; agar, 15 gm.; distilled water, 1,000 cc.

portion of the temperature-growth curve represents the average diameter attained by the fungus on 40 plates. Growth at each of the temperatures 95° and 100° is based upon the average of 10 plates, while at 45° and 50° it is represented by the average of 35 and of 34 plates, respectively. Six different isolates were used at various times during the 4 experiments.

There was no growth of the fungus at 45° F. in 4 days, 1 week, or 2 weeks. Growth was slight at 50° in 4 days, and moderate in 1 week and 2 weeks. At 55° growth was moderate in 4 days, and extensive in 1 week and 2 weeks.

In later tests slight growth was observed in 1 week at 47.5° F., so that the minimum temperature for growth under the conditions of the test lies somewhere in the range of 45° to 47.5°.

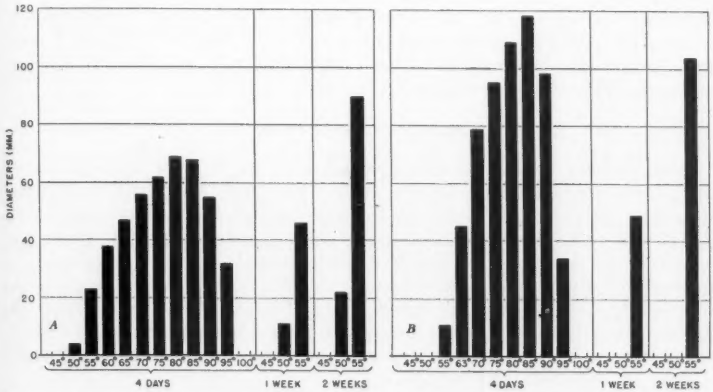

FIGURE 5.—*A*, Temperature relations of *Phytophthora capsici* from Winter Queen watermelon; average diameter of colonies developed on potato-dextrose agar in 4 days, 1 week, and 2 weeks. *B*, Temperature relations of phytophthora rot as determined by average diameter of lesions developed on Winter Queen watermelon in 4 days, 1 week, and 2 weeks.

Growth in 4 days increased fairly regularly with increase of temperature from 50° to 80° F., where maximum growth occurred. However, growth was nearly as great at 85° as at 80°. Above 85° growth fell off rapidly with increase of temperature, and no growth occurred at 100° in 4 days. These results are in accordance with data presented by Tompkins and Tucker (*15*); their isolates from Honey Dew melons grew most rapidly at 25° and 30° C., and all made some growth at 35°.

The effect of temperature upon the rate of development of phytophthora rot was determined by three experiments in which artificially inoculated Winter Queen watermelons were held at various temperatures within the range of 45° to 100° F. The methods used consisted of washing the melons with soap and water, drying them, and placing them in the desired temperature chamber. Twenty-four hours later they were removed, washed thoroughly with 95-percent ethyl alcohol, dried, and inoculated at four points per melon by inserting generous portions of a several-day-old agar culture in shallow wounds made with a sterile scalpel into and through the rind. The

number of melons used varied somewhat, so that the total number of lesions measured in order to determine the average diameter for each temperature point were as follows: At 45°, 40 lesions; at 50°, 42; at 55°, 56; at 63°, 48; at 70°, 36; at 75°, 36; at 80°, 36; at 85°, 36; at 90°, 14; at 95°, 4; at 100°, 8. The results are shown in figure 5, *B*.

No decay developed at 45° or at 50° F. in 4 days. Some decay occurred at 55° in 4 days; from 55° to 85° the average diameter of the lesions increased regularly with increase in temperature. Maximum decay development occurred at 85°, although the lesions were nearly as large at 80°. Decay development at 90° was about the same as that at 75°, but it fell off materially at 95°, and no decay occurred in 4 days at 100°.

Decay development at 55° F. was moderate in 1 week and advanced in 2 weeks. When the usual method of inoculation was used no decay developed at 50° even in 2 weeks. However, when melons with definite lesions of phytophthora rot were held at 50° for 1 week and 2 weeks the lesions continued to enlarge moderately. In no case was there any decay development at 45°.

PENETRATION STUDIES

Observations made on commercial lots of Winter Queen watermelons showed that in many crates phytophthora rot was more abundant at points of contact between adjacent melons than elsewhere over the melon surface. This suggested the possibility of spread from diseased to healthy melons within the container. A number of small-scale experiments were therefore made for the purpose of determining whether the pathogen could penetrate the apparently unbroken skin of the melon and whether or not the decay would spread from melon to melon under conditions simulating those existing in commercial practice.

Melons selected for their general freedom from blemishes and scratches were washed with soap and water, dried, washed with 95-percent ethyl alcohol, and again allowed to dry. Small plantings of potato-dextrose agar cultures of the fungus previously isolated from decaying Winter Queen watermelon were then placed at isolated spots on the melon surface where the rind appeared to be entirely free of blemishes or injuries of any sort. These were covered with inverted sterilized wide-mouth vials,[9] which were fastened to the melon by means of melted paraffin wax. In some instances a piece of sterilized cotton moistened with sterilized water was laid over the agar-mycelium planting before the vial was sealed on. In others no cotton was used, but the inside of the vial was moistened with sterile water. Suitable checks, consisting of sterile agar plantings, were similarly set up.

In 1 such test 3 melons were each inoculated at 10 points with 1 of 3 different isolates. Three days later 90 percent of infection had occurred with each of 2 isolates, and 70 percent with the third isolate. No decay developed on the checks.

A second test consisted of 19 inoculations, of which 17, or 89 percent, resulted in infections with phytophthora rot.

In a final test of a similar type, in which moistureproof cellophane firmly secured around the edges was substituted for the glass vials, all but 1 out of a total of 15 inoculations were positive within 6 days.

[9] Lipless thick-walled glass bottles 25 mm. in diameter and 70 mm. in length, of the type known as shell vials.

Evidences of infection were visible within 2 or 3 days at room temperature.

The results of the three tests indicate that *Phytophthora capsici* is capable of penetrating the rind of Winter Queen watermelons in the absence of visible scratches or injuries in the rind.

In the first test made to determine whether spread of decay occurs within the crate, 5 healthy melons and 3 melons bearing advanced lesions of phytophthora rot were laid together within a large basket in such a manner that 15 points of contact were made between healthy and diseased rind. The healthy melons bore no fresh bruises nor wounds. They were not washed, nor were their surfaces sterilized. All were held at a mean daily temperature of about 70° to 75° F. for 4 days. At the end of that time phytophthora rot was well advanced on the previously healthy rind at each of the 15 places where contact had been made with infected melons. At the start of the test the decay lesions on the 3 infected melons were more moist than the earlier stages of decay as ordinarily found in commercial packages. Moreover, all 8 melons were packed together somewhat more tightly than in commercial packing.

Three tests were therefore made, in each of which only early to moderately advanced stages of decay were used as the source of inoculum and in which the diseased and healthy melons were packed in crates with pressure of melon against melon approximating that found in commercial crates. In all 3 tests the healthy melons were washed and dried, and the areas of healthy rind exposed to the decay lesions were selected for freedom from fresh scratches and wounds. The arrangement was such that the diseased melons made 10 contacts with 8 sound melons. The crates were held at room temperatures approximating a daily mean of 72° F. for 6 days. Phytophthora rot had by that time spread to the healthy melons at all 10 points of contact. Similarly, in a repetition of this test with other melons, there was infection at all 10 points of contact in a 4-day period. In a final test of this sort 20 contacts were made between healthy and diseased melons. After 5 days phytophthora rot had spread to the healthy melons at 18 points.

The four tests show clearly that phytophthora rot can spread from melon to melon within the container, and that by the end of 4 days at temperatures near 70° F. lesions are developed that are of considerable economic importance.

HOST RANGE

Inoculations with the six isolates of *Phytophthora capsici* were made by the method used by Tompkins and Tucker (*15*). All isolates were found to be similar in pathogenicity and proved to be capable of penetrating unwounded tissues and causing rots of the fruits of pepper, tomato, apple, cantaloup, cucumber, and squash, and of carrot roots. They infected wounded fruits of orange and lemon, and wounded potato tubers. The symptoms produced on the various commodities agreed very closely with those reported by one of the writers (*17*), using isolates from pepper, and by Tompkins and Tucker (*15*), using isolates from Honey Dew melons.

Inoculations of turnip and radish roots and of onion bulbs were negative.

The pathogenicity of *Phytophthora capsici* for pepper plants has been used as a criterion for the identification of the species (*17*). California Wonder pepper plants were therefore inoculated with the six watermelon isolates by placing a bit of mycelium from oatmeal-agar cultures in a small slit in the stem near the tip. Twenty-four plants were thus inoculated, each isolate on four plants. All isolates proved virulently pathogenic, killing the terminal growing region and causing browning and collapse of the stems 4 to 6 inches below the point of inoculation within 6 days (fig. 6). Inoculations of young pepper plants, made by mixing cultures with the soil, caused wilting and death of all plants within 25 days. The watermelon isolates were as pathogenic for pepper plants as were isolates from pepper and Honey Dew melon.

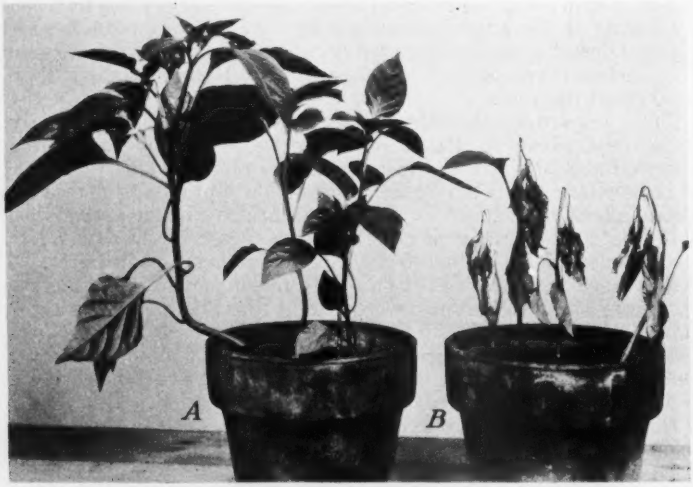

FIGURE 6.—California Wonder pepper plants: *A*, Wounded but not inoculated; *B*, inoculated with *Phytophthora capsici* isolated from Winter Queen watermelon. Photographed 6 days after inoculation.

CONTROL

As has been pointed out, the organism that causes the phytophthora rot of Winter Queen watermelons has been likewise responsible for a field decay of Honey Dew melons in California. There the disease was found restricted to areas of irrigated fields in which excessive water accumulated as a result of poor drainage. In Colorado the same fungus was reported (*1*) on peppers in the vicinity of Rocky Ford in 1931 and at Canon City in 1932. The disease was most prevalent in heavy wet soils. What is apparently the same fungus was also reported (*7*) as the cause of a field decay of cucumber fruits at Rocky Ford in 1936.

The causal fungus is soil-borne and has been shown (*1*) to be able to remain alive in the soil of coldframes for at least 2 years. It would therefore seem advisable to avoid planting Winter Queen watermelons in fields where infections by *Phytophthora capsici* have recently occurred on pepper, cucumber, or other crops.

Although no information is available on the occurrence of the disease in the fields of Colorado, where the infected Winter Queen watermelons originated, the wide variations in the percentage of melons affected in different shipments and during different seasons suggest that environmental factors in the field play an important role in determining the importance of the disease during marketing. The presence of excessive soil moisture is undoubtedly an important factor, and where this condition prevails it would be well to determine, prior to the time of picking, whether the decay is present on fruits in the field. If so, then care may be exercised in harvesting to avoid shipping melons from such areas in the field. However, should the fruit rot be found rather generally distributed, even in small amount, over the field, it should be recognized that the chances for serious development of decay during the transit and marketing period are greatly increased.

The prevalence of unusually wet conditions shortly before harvest may result in extensive infection in fields where the pathogen is present and active. Melons that have been only recently infected may show no evidence of the disease at the time of packing. Under temperatures prevailing in cars shipped under ventilation without ice, such melons soon become extensively rotted and may readily serve as centers for spread of the decay to adjacent melons in the containers. From the results of the temperature studies it would appear that reduction of carrying temperatures to 50° F. through the use of refrigeration would materially retard decay and that maintenance of a temperature of 45° would completely arrest the development and spread of the decay during the transit period.

SUMMARY

A phytophthora rot of Winter Queen watermelons grown in Colorado was first observed on the New York City market in 1935.

During the four-season period of observation it was found that the disease occurred sporadically, but that when present in a given carlot it might affect from 5 percent or less to nearly all of the melons.

The causal organism was found to be *Phytophthora capsici* Leon.

The optimum temperature for growth of the pathogen in culture was in the range of 80° to 85° F. The maximum temperature (for growth in 4 days) lay between 95° and 100°. The minimum temperature for growth of the organism lay between 45° and 47.5°. Growth did not occur at 45° in 19 days.

The development of decay on inoculated Winter Queen watermelons was greatest at 85° F. At temperatures in the range of 63° to 70°, lesions attained a diameter of 45 to 79 mm. (approximately 2½ to 3 inches) in 4 days. At 50° old lesions continued to enlarge slowly, but new lesions were not apparent in 14 days. No decay developed at 45° in 14 days.

The causal fungus was found to be able to penetrate the apparently unbroken skin of Winter Queen watermelons. Tests indicated that at favorable temperatures the decay can readily spread w thin the container from diseased to healthy melons.

Tests showed that the causal fungus was pathogenic for pepper plants and that it could penetrate and rot unwounded fruits of pepper, tomato, apple, cantaloup, cucumber, and squash, and unwounded

carrot roots. The fungus also produced a rot of wounded orange and lemon fruits and wounded potato tubers.

Suggestions for control, based upon certain field and harvesting practices, are presented. Reduction of transit temperatures to 50° F. should greatly retard decay development, and the maintenance of a temperature of 45° should completely arrest the development and spread of decay during transit.

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THE USE OF ASCORBIC ACID (CRYSTALLINE VITAMIN C) AS A SUBSTRATE IN OXIDASE MEASUREMENTS[1]

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INTRODUCTION

During storage the physiological changes in fruits are katabolic, and oxidative processes are doubtless dominant. A study of these processes is of importance from the standpoint of fruit behavior and particularly with reference to physiological diseases such as soft scald and break-down of apples and core break-down of pears. A few degrees difference in storage temperature may mean either severe losses or fruit comparatively free from disease. Thus, at 30° or 32° F. certain varieties of apples may develop severe soft scald, whereas at 36° this disease very seldom develops. However, at the higher temperature the period of profitable storage is shortened owing to more rapid ripening. These results indicated that a study of the oxidizing enzymes might be of considerable importance for an understanding of the basic causes of this type of disease and in turn might lead to methods for its control. Preliminary tests indicated that the difference in oxidase activity of fruit stored at 32° and 36° might be small. A more sensitive method than had previously been available for measuring small changes in enzyme activity was needed, and the present study was therefore undertaken.

Oxidase activity may be determined colorimetrically (*16, p. 234*),[2] volumetrically (*4*), or iodometrically (*3*). Of these three processes, the last is in many respects the most satisfactory. In Guthrie's (*3*) iodometric method the oxidizable substrate is prepared by heating glucose with a dilute sodium hydroxide solution. The glucose is broken down into numerous derivatives, some or all of which reduce iodine in acid solution. These carbohydrate degradation products are unstable and lose their oxidizing power in the presence of air, and their oxidation is catalyzed by plant juices, i. e., by oxidase.

When glucose is heated with dilute alkali, a mixture of various decomposition products is formed. It is conceivable that some of these carbohydrate derivatives may require different degrees of oxidation before their iodine-reducing power is destroyed, while other products in this mixture may possess no iodine-reducing power or may even have an inhibitory action on the oxidase enzyme. For this reason it would seem desirable to have a pure substrate in which the oxidizable material would be uniform and other products would not be present as a possible source of interference with the enzyme action.

In the quantitative determination of ascorbic acid in plant tissues it is necessary to inactivate the plant oxidase to prevent destruction of the acid. Szent-Györgyi (*13*) noted the reducing power of plant juices and identified the reducing substance as hexuronic acid. This

[1] Received for publication April 1, 1939.
[2] Italic numbers in parentheses refer to Literature Cited, p. 98.

was later termed ascorbic acid and is now available in crystalline form. Ascorbic acid, then, is a definite chemical compound; it may be obtained in pure form; it is readily oxidized by the oxidase enzyme; and the amount of oxidation may be determined by iodine reduction in acid solution. It therefore appeared likely, and preliminary tests indicated, not only that ascorbic acid could be used as a substrate for oxidase measurements but that it possessed distinct advantages over the glucose-derivative substrate as prepared by Guthrie.

This paper presents the results of a study in which ascorbic acid (crystalline vitamin C) is used as a substrate for oxidase measurements in comparison with the glucose-derivative substrate of Guthrie. The influence of concentration of the enzyme, of the ascorbic acid, of the acidity, and of various other factors has received attention.

MATERIALS AND METHODS

The enzyme preparation consisted of the pressed juice in most cases. The tissue to be used was ground in a food chopper with a nut-butter attachment, and the juice was squeezed through two thicknesses of cheesecloth. The juice was then filtered through double cheesecloth, without pressure, to remove any tissue forced through at the first filtering.

The method of measuring oxidase activity was similar to that described by Guthrie (*3*) except that 25 cc. of a water solution of ascorbic acid crystals (cevitamic acid, Merck) was used, usually in concentrations of 0.1 to 0.4 percent, as a substrate in the Van Slyke-Cullen aeration tubes, instead of the heated glucose solution of Guthrie. From 1 to 10 cc. of the juice or extract containing the enzyme was added to each tube, the amount depending on the oxidase content. The boiled preparation was used as a blank. Whenever the enzyme-substrate solution foamed badly, 5 drops of paraffin oil were added to each tube. Uniform reaction conditions were obtained by drawing air for 1 hour, at the rate of 8 liters per hour, through a constant-temperature bath held at 25° C. After aeration the solutions were transferred with 50 cc. of water to 500-cc. Erlenmeyer flasks containing 25 cc. of 10-percent trichloroacetic acid. Twenty cubic centimeters or more of N/50 iodine in N/10 potassium iodide was then added, and after standing for 30 minutes the solution was titrated with N/100 sodium thiosulfate. The difference in titration between the boiled and the unboiled sample gave a measure of the oxidase activity. When several samples are aerated simultaneously, care should be taken to keep the iodine oxidation-reduction period uniform.

EXPERIMENTAL WORK

EFFECT OF THE HYDROGEN-ION CONCENTRATION OF THE SUBSTRATE ON OXIDASE ACTIVITY

Water solutions of ascorbic acid are acid in reaction. Falk (*2, p. 98*) states that the optimum pH value for vegetable oxidase activity is from 7 to 10. This is more alkaline than most plant juices. Apple juice has a pH value of approximately 4. With potato juice and a sugar-derivative substrate, Guthrie (*3*) suggests a pH value of 6.5 for oxidase measurement.

In order to determine whether the acidity of the ascorbic acid solution interfered seriously with the oxidase activity, this substrate

was adjusted to various acidities with N/10 sodium hydroxide and buffered with Clark and Lubs buffer solutions. Equal parts of the substrate and of buffer solutions were used and adjusted to a constant volume and concentration. The oxidase activity of apple juice was then determined at various acidities. The results are given in table 1, column 2.

The substrate with a pH value of 4.0 was more acid than is usually recommended for optimum oxidase activity, but the use of sodium hydroxide and buffer solutions to give a pH value as high as 8.0 did not increase the enzyme activity. The fact that there was such a wide difference between the check (unbuffered substrate) and the sample buffered to pH 4.0, both of which were near the same acidity, suggested that the buffer chemicals were interfering with the enzyme action. This proved to be true, as is shown in table 1, column 3, when the substrate was brought to the desired pH value with N/10 sodium hydroxide without addition of buffer. With the acidity thus reduced enzyme activity was again less than in the unneutralized substrate.

TABLE 1.—*Effect of the hydrogen-ion concentration of the substrate on oxidase activity*

Ascorbic acid substrate (pH) [1]	Oxidase activity (N/100 $Na_2S_2O_3$)		
	Apple juice (substrate buffered)	Apple juice (substrate unbuffered)	Potato juice (substrate unbuffered)
(1)	(2)	(3)	(4)
	Cc.	*Cc.*	*Cc.*
Check [2]	6.2	8.5	7.1
4.0	.7	8.5	7.1
5.0	2.6	6.6	6.5
6.0	3.8	6.1	7.3
7.0	2.7	5.2	7.1
8.0	1.5	5.2	7.2

[1] N/10 NaOH added to secure pH value desired.
[2] 0.1-percent water solution, pH value approximately 4.0.

Since apple juice has a pH value approximately equal to a 0.1-percent solution of ascorbic acid, it was thought that an enzyme preparation less acid than apple juice might behave differently in regard to the acidity of the substrate. Although repeated tests with potato juice having a pH value of 6.2 did not always show increased oxidase activity with the more alkaline substrates, the effect of adjustment did not appear to be harmful. The results of one of these tests are given in table 1, column 4. These results with apple and potato juice might indicate that the optimum pH value for oxidase activity is near that of the original material; i. e., apple near pH 4.0 and potato near pH 6.2. The failure of the potato juice to show definitely greater activity with the adjusted substrates may be due to the injurious effect of the sodium hydroxide on the enzyme, offsetting the advantages of increase in alkalinity.

EFFECT OF CONCENTRATION OF SUBSTRATE ON OXI DASE ACTIVITY

The effect of concentration of the substrate on the oxidase activity of apple juice is shown (table 2) under conditions in which the concentration of the ascorbic acid ranged from 0.1 to 0.4 percent. Two cubic centimeters of apple juice was used at each concentration.

Differences in concentration of the substrate from 0.1 to 0.3 percent of ascorbic acid had little effect on the enzyme activity, whereas 0.4 percent was strong enough to cause injury to the enzyme (table 2, column 2). Guthrie (*3*) has stated that boiled juice has a protective effect on the oxidase enzyme. When 8 cc. of boiled juice was added to 2 cc. of unboiled juice and the test repeated, no injury at 0.4 percent was evident (table 2, column 3). In a later test, with onion juice very weak in oxidase, a 0.3-percent ascorbic acid solution showed slight injury to the enzyme.

TABLE 2.—*Effect of concentration of substrate on the oxidase activity*

Concentration of substrate (percent)	Oxidase activity (N/100 $Na_2S_2O_3$)	
	Enzyme unprotected	Enzyme protected [1]
(1)	(2)	(3)
	Cc.	*Cc.*
0.1	9.9	12.8
0.2	9.9	13.0
0.3	9.9	13.0
0.4	8.0	13.0

[1] Enzyme protected by the addition of 8 cc. of boiled juice to 2 cc. of unboiled juice.

For routine analysis, where the titration difference between the boiled and the unboiled juice is not more than 15 cc. of N/100 thiosulfate, a substrate concentration of 0.1 percent of ascorbic acid has been found satisfactory. Since 1 cc. of N/100 thiosulfate is equivalent to 0.88 mg. of ascorbic acid (*8*), a titration of 15 cc. would indicate that 13.20 mg. of ascorbic acid had been oxidized by the enzyme. The amount oxidized by the blank varies with the product and with the amount of boiled material used: 2 cc. of boiled apple juice oxidized 1.85 mg. of ascorbic acid during aeration; a difference of 15 cc. in titration would indicate that approximately 60 percent of the substrate had been oxidized.

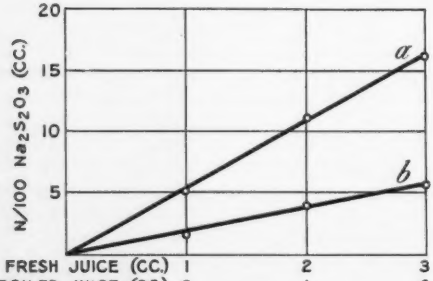

FIGURE 1.—Effect of concentration of the enzyme on the oxidase activity of apple juice: *a*, Relatively strong oxidase solution; *b*, relatively weak oxidase solution

EFFECT OF CONCENTRATION OF ENZYME ON OXIDASE ACTIVITY

When the protective action of the juice is kept constant by the addition of boiled juice, the oxidase activity varies directly with the concentration of the enzyme. This is shown in figure 1, where the activity of apple juice relatively strong and weak in oxidase is plotted.

EFFECT OF THE SUBSTRATE AND ITS OXIDATION PRODUCTS ON THE ENZYME

The product of a reaction often interferes with the action of the enzyme, and as a result the concentration of active enzyme may change during the course of the reaction. For instance, when 2-cc. samples of Winesap apple juice were aerated for 20, 40, and 60 minutes, the oxidase activity was 3.4, 6.7, and 9.7 cc., or 3.4, 3.3, and 3.0 cc. for the three 20-minute intervals, respectively. This reduction in enzyme activity may be due to the action of the substrate or to the products formed by the reaction between the enzyme and substrate. Guthrie (*3*) noted that this destructive action was reduced by the addition of boiled juice to the unboiled juice. To determine whether the ascorbic acid substrate was as injurious to the enzyme as the glucose-derivative substrate, a test was made in which both ascorbic acid and glucose-derivative substrate were used. In each case the quantity of unboiled juice was kept constant while the quantity of boiled juice was varied. The results are shown in table 3.

It is evident that the boiled juice was more effective in increasing oxidase activity with the heated glucose-derivative substrate than with the ascorbic acid substrate. Also the difference between 2 and 4 cc. of boiled juice was much greater in the glucose-derivative substrate than in the ascorbic acid substrate. Conversely, it may be stated that the heated glucose-derivative substrate or the products of its oxidation are more destructive to the enzyme than is the ascorbic acid substrate or its oxidation products. The substrate, reduced or oxidized, should be as free as possible from such destructive substances.

TABLE 3.—*Effect of different substrates on the oxidase activity of apple juice when various proportions of protective boiled juice are used*

Enzyme preparation	Activity of apple juice on—			
	Ascorbic acid substrate		Glucose-derivative substrate	
	Oxidase activity (N/100 $Na_2S_2O_3$)	Increase over unprotected juice	Oxidase activity (N/100 $Na_2S_2O_3$)	Increase over unprotected juice
	Cc.	*Percent*	*Cc.*	*Percent*
2 cc. of unboiled juice, no boiled juice	7.0		4.2	
2 cc. of unboiled juice + 2 cc. of boiled juice	9.4	34.3	6.3	50.0
2 cc. of unboiled juice + 4 cc. of boiled juice	10.0	42.8	7.8	85.7

Johnson and Zilva (*5*) have shown that certain plant enzymes oxidize catechol in catechol-ascorbic acid mixtures but only after the ascorbic acid is all oxidized. This would indicate that ascorbic acid is more readily oxidized than catechol. If increasing amounts of an enzyme preparation are added to a constant amount of ascorbic acid substrate until an excess of enzyme is present, a slight excess of enzyme shows no increase in oxidase activity and no difference in the color of the solution. However, if an additional excess is added, further visible oxidation takes place and darkening results, the amount of darkening depending roughly on the excess enzyme added. It should be noted that visible darkening does not take place until all of the ascorbic acid is oxidized. According to Onslow (*10, p. 122*) oxidase

is made up of three components, one of which is an aromatic substance containing an orthodihydroxy grouping such as that in catechol. The darkening may be due to the oxidation of this aromatic compound. This would seem to agree with Johnson and Zilva's results, in which the catechol, in ascorbic acid-catechol mixtures, was not oxidized until all of the ascorbic acid had been oxidized.

The direction of the ascorbic acid oxidation appears somewhat obscure. Ascorbic acid is generally considered to be first oxidized to dehydroascorbic acid, which is approximately equal to ascorbic acid in antiscorbutic potency. According to Borsook et al. (*1*), dehydroascorbic acid in aqueous solutions undergoes a spontaneous irreversible change at ordinary temperatures if the hydrogen-ion concentration is less than pH 4, forming a stronger acid and a more powerful reducing agent than ascorbic acid itself. However, this change is independent of the presence of air or oxidizing agents; consequently it is not an oxidation. Moll and Wieters (*9*) found that pure aqueous solutions of dehydroascorbic acid are far less stable than ascorbic acid solutions, and that the antiscorbutic property is lost more quickly than the property of being regenerated by hydrogen sulfide. This degradation product is an ascorbic-acid-like substance with a characteristic reductive action on iodine, silver nitrate, and dichlorphenolindophenol, but without antiscorbutic properties. In order to determine whether the products of the ascorbic acid oxidation were reducing the iodine in a similar manner to the unoxidized ascorbic acid the following study was made, in which the enzyme was permitted to act on the substrate over a long period of time and in which the oxidation products were allowed to stand in strongly acidified solutions. The results are as follows:

Lot No. and treatment:	Oxidase activity (cc. N/100 $Na_2S_4O_3$)
(1) Check, 2 cc. of enzyme material, aerated 1 hour; oxidase determined by usual procedure	2.9
(2) 2 cc. of enzyme material, aerated 1 hour and then let stand for 17 hours at 27° C. before adding acid and iodine and determining the activity	4.6
(3) 2 cc. of enzyme material, aerated 1 hour; then 25 cc. of 10-percent trichloroacetic acid added and let stand 17 hours at 27° C. before iodine added and activity determined	3.0
(4) 10 cc. of enzyme material, aerated 1 hour, but let stand 17 hours at 27° C. before acid and iodine added and activity determined	[1] 23.6
(5) 10 cc. of enzyme material, aerated 1 hour; then 25 cc. of 10-percent trichloroacetic acid added and let stand 17 hours at 27° C. before iodine added and activity determined	[1] 20.0

[1] All of the substrate was oxidized in lots 4 and 5. The apparent difference in activity was due to difference in the blanks. The blank in lot 5, acidified following aeration, was greater than the blank in lot 4. The thiosulfate titration was practically the same in all tubes containing the enzyme in the 2 lots.

From the above data, it appears that the degradation products did not react with the iodine as the ascorbic acid did even when the enzyme was allowed to act for 18 hours or when the oxidized substrate was allowed to stand in a strongly acidified solution for 17 hours. Lot 1 was a comparatively weak enzyme solution and oxidized but little of the substrate when determinations were made by the usual procedure. Lot 2, in which the enzyme was allowed to act without aeration for an additional 17 hours, showed 58 percent more of the substrate oxidized than lot 1. Lot 3 had the enzyme killed at the end of aeration and, as would be expected if the oxidized substrate

did not react with the iodine, showed practically the same activity as lot 1. Lots 4 and 5, containing 10 cc. of the enzyme, oxidized all the substrate in less than 1 hour as was shown by a brownish discoloration at the end of the aeration. Lot 4 showed increased darkening after 18 hours and was distinctly darker at the end of the period than lot 5, in which the enzyme was killed at the end of aeration. Yet no lot gave any indication that the oxidized substrate was reducing the iodine as occurs in the case of ascorbic acid. The apparent difference in activity between lots 4 and 5 is due to a difference in the blanks; the acidified blank gave a higher thiosulfate titration than did the one that remained unacidified overnight. It should be recalled that the thiosulfate titration is a measure of the excess iodine added after the enzymic oxidation of the ascorbic acid; it is directly proportional to the enzyme activity and inversely proportional to the ascorbic acid remaining in the substrate.

Even though the reducing substance formed from dehydroascorbic acid is present in the oxidized substrate, it still would be ineffective in reducing the iodine. Borsook et al. (*1*) have shown that it is inactive as a reducing agent below pH 4. The acidified substrate, containing 25 cc. of 10-percent trichloroacetic acid to which the iodine is added, is considerably lower than pH 4. At no time has there been any indication of a reducing substance being formed in the oxidized substrate.

ENZYMIC VERSUS METALLIC OXIDATION OF THE ASCORBIC ACID

It is well known that copper can catalyze the oxidation of ascorbic acid. Stotz et al. (*12*) found that the copper in the so-called "ascorbic acid oxidase" from squash and cauliflower was sufficient to account for the oxidation credited to the enzyme and that the greater part of the copper could be recovered in the coagulated protein upon heat inactivation. In the present work practically the same results were obtained whether the enzyme was killed by heating or by the addition of 10-percent crystalline trichloroacetic acid to the juice. Other metals also may catalyze the oxidation of ascorbic acid (*7*). That grinding the tissue through the food chopper had no effect on the rate of oxidation of the ascorbic acid was shown by using ripe peaches in which the juice could be hand-pressed without cutting or grinding. Hand-pressed juice gave the same activity as did the juice from similar tissue ground in the food chopper before pressing.

That the oxidation of the ascorbic acid was due to an oxidizing enzyme and not to copper or other metals present in the juice is shown by the results of an experiment (table 4) in which the oxidase activity of the same enzyme preparations was determined at various intervals of time. The enzyme solutions were held in stoppered Erlenmeyer flasks at room temperature throughout the test. If the oxidation were due to copper, then with the copper content of the juice remaining constant the rate of oxidation of the substrate should also remain constant; but if the oxidation were due to an enzyme, the enzyme gradually would be destroyed with standing, and the rate of oxidation should decrease. The data in table 4 show that the ability to oxidize the substrate diminishes with time. This would indicate that the oxidation is enzymic in character.

Other indications that the oxidation is due to an enzyme and not to copper that might be present in the juice are given in table 3 and

on page 93. The addition of boiled juice (table 3) would not be expected to exert a protective action on the copper but might be expected to do so with enzymes. Also, the copper content being constant, the amount of oxidation would be expected to remain constant during equal intervals of time. That such is not the case is shown on page 93.

COMPARATIVE EFFECT OF DIFFERENT SUBSTRATES ON THE OXIDASE ACTIVITY OF VARIOUS PLANT PRODUCTS

The relative oxidase activity of various plant products was determined with ascorbic acid substrate and with the glucose-derivative substrate prepared as recommended by Guthrie (*3*). The results are given in table 5.

TABLE 4.—*Effect of time on oxidase activity*

Time elapsed after preparation	Oxidase activity (N/100 $Na_2S_2O_3$) of—	
	Delicious apple juice	Golden Delicious apple juice
	Cc.	*Cc.*
2 hours	9.5	8.0
26 hours	5.7	6.2
45 hours	2.1	3.8
15 days [1]	0	.5

[1] Slight growth of mold in solutions at this time.

TABLE 5.—*Oxidase activity of various plant products as influenced by the kind of substrate*

Source of enzyme		Oxidase activity (N/100 $Na_2S_2O_3$)		Source of enzyme		Oxidase activity (N/100 $Na_2S_2O_3$)	
Material	Quantity	Ascorbic acid substrate	Glucose-derivative substrate	Material	Quantity	Ascorbic acid substrate	Glucose-derivative substrate
	Cc.	*Cc.*	*Cc.*		*Cc.*	*Cc.*	*Cc.*
Winesap apple juice (fruit)	2	7.1	3.9	Cantaloupe juice (melon)	10	0.7	0.0
Red Rome apple juice (fruit)	5	8.1	5.5	Lettuce juice (head)	2	6.2	2.0
Bosc pear juice (fruit)	2	2.3	1.0	Cucumber juice (fruit)	2	26.1	15.5
Bartlett pear juice (fruit)	2	4.0	1.7	Watermelon juice (red meat)	10	0	0
Peach juice (fruit)	5	12.8	5.7	Orange juice (fruit)	10	0	0
Plum juice (fruit)	5	15.4	7.7				
Potato juice (tuber)	2	24.6	15.5	Average		6.52	3.28
Onion juice (bulb)	5	1.4	1.1				
Carrot juice (root)	5	8.3	1.3	Average increase over glucose-derivative substrate		*Percent* 98.7	
Radish juice (root)	5	0	0				
Cabbage juice (head)	2	1.6	.8				
Water suspension of—							
Dried cherry bark	2	1.2	0				
Do	4	2.0	.4				
Fresh cherry bark	5	2.4	.3				

It is interesting to note that the average oxidase activity of these various plant products was 98.7 percent greater with the ascorbic acid substrate than with the sugar-derivative substrate. This greater activity is especially important where the amount of enzyme activity is small. In some cases where no activity was evident with the glu-

cose-derivative substrate, definite activity was shown with the ascorbic acid substrate. That the ratio of activity for the substrates did not remain constant with different enzyme preparations was probably due to the varying amounts of the injurious substances in the sugar-derivative substrate or to the relative immunity of the various preparations to injury from such substances. Table 3 shows that the sugar-derivative substrate was more injurious to the enzyme than was the ascorbic acid substrate.

DISCUSSION

In the determination of ascorbic acid in plant tissues by dichlorphenolindophenol or other chemical means it is often necessary to inactivate the plant oxidase to prevent the ascorbic acid from being oxidized to dehydroascorbic acid, which is biologically active but does not react with the dichlorphenolindophenol dye. Plants differ considerably in the rate at which this change takes place. Stone (*11*) concluded that it was a question of ascorbic acid oxidase. If the oxidase was present the plant lost the indophenol-reducing power on mincing; if the oxidase was absent the indophenol-reducing power remained.

Several workers (*5*, *6*, *11*, *13*, *14*, *15*) have reported the presence of an ascorbic acid oxidase that can oxidize ascorbic acid. Szent-Györgyi (*13*), the first to note this property, states that the hexuronic acid (ascorbic acid) oxidase is not a single enzyme but apparently involves a number of catalysts. Tauber et al. (*15*) isolated an ascorbic acid enzyme from the pericarp of Hubbard squash which gave none of the color reactions that other oxidases give with such reagents as benzidine, guaiacol, pyrogallol, catechol, phloroglucinol, resorcinol, naphthoresorcinol, or vanillin. Nor did it affect glutathione, cysteine, tyrosine, adrenalin, or glucose boiled with alkali, and its kinetics indicated a single enzyme. However, they found practically no ascorbic acid, reduced or oxidized, in the fruit, so the role of this enzyme in the physiology of the plant remained obscure.

Copper catalyzes the oxidation of ascorbic acid very readily, and precautions must be taken to prevent it from oxidizing the ascorbic acid in the dichlorphenolindophenol method of analysis (*7*). Stotz et al. (*12*) studied the role of copper in the ascorbic acid oxidase from squash and cauliflower and reported that the copper content was sufficient to account for the observed catalysis and that the greater part of the copper in such preparations was found in the coagulated protein upon heat inactivation. Additional results strengthened the indication that copper was responsible for the oxidation of the ascorbic acid. Stotz et al. (*12*) denied any need for using such terms as "ascorbic acid oxidase," "vitamin C oxidase," or "hexuronic acid oxidase," at least in the sense that they have been used in the past.

However, it is generally recognized, and the work reported herein emphasizes, that there are certain plant enzymes that are capable of oxidizing ascorbic acid. These same enzyme preparations oxidize the degradation products formed when glucose is heated with an alkali, and thus seem to be the same as or closely associated with indophenol oxidase (*3*). Indirectly, these results would support Stotz et al. (*12*) in their belief that no special "ascorbic acid oxidase" is necessary to account for the oxidation of ascorbic acid.

SUMMARY

The use of ascorbic acid as a substrate in oxidase measurements is suggested. The effects of various factors, including the pH value of the substrate, concentration of the substrate, and concentration of the enzyme, were determined. Ascorbic acid was shown to be less injurious to the enzyme than a glucose-derivative substrate. In the case of numerous plant products, the oxidase activity with an ascorbic acid substrate averaged 98.7 percent greater than with a glucose-derivative substrate. This greater sensitivity should permit measurements of smaller changes in oxidase activity of fruits in storage. It should find application in investigations not only of soft scald but also of certain other physiological diseases wherein oxidative changes may be a causative factor. Data are included to show that the oxidation of the ascorbic acid is enzymic and not metallic.

Ascorbic acid substrate possesses the following advantages over a glucose-derivative substrate: (1) Ascorbic acid is a definite chemical compound available in pure form; (2) it is more readily oxidized, enabling the investigator to note smaller differences in enzyme activity; (3) it is water-clear in solution, facilitating titration; (4) it is less injurious to the enzyme; and (5) its use as a substrate saves time, since ascorbic acid is readily soluble in water and a fresh solution may be prepared in a few minutes.

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EFFECTS OF VARIOUS AMOUNTS OF NITROGEN, POTASSIUM, AND PHOSPHORUS ON GROWTH AND ASSIMILATION IN YOUNG APPLE TREES [1]

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INTRODUCTION

During the past 25 years it has become generally recognized in the United States that most commercial apple orchards require rather liberal applications of nitrogenous fertilizers to maintain them in optimum production. Experimental results relative to the need for potassium and phosphorus have been less conclusive. Some investigators have reported definite benefits from the use of potassium (*10*, *14*),[2] while many others have observed no beneficial results from its use (*2*, *9*). Since potassium-deficiency symptoms rarely have been observed on apples in this country, the assumption is generally made that soils contain a sufficient amount of this element to meet the needs of apple orchards.

It is generally agreed that fruit soils of the United States have a sufficient supply of available phosphorus to meet tree requirements. Although there are a few instances on record that suggest a benefit from phosphorus (*8*), there is no evidence that apple orchards usually respond to applications of phosphorus.

Several investigators have estimated the relative intake of nitrogen, phosphorus, and potassium by apple trees. Van Slyke, Taylor, and Andrews (*13*) estimated the proportion for bearing trees as 4 parts nitrogen (N_2), 1 part phosphorus pentoxide (P_2O_5), and 4 parts potassium oxide (K_2O). This would approximate 9 parts nitrogen, 1 part phosphorus, and 7 parts potassium. Heinicke (*6*) reports a relationship for heavily bearing trees of 3.9 nitrogen, 0.5 phosphorus, and 3.5 potassium, or a ratio of approximately 8 : 1 : 7. Thomas (*12*) reported for nonbearing apple trees receiving an N–P–K fertilizer treatment in cultivation a ratio of 5.72 N_2, 1.0 P_2O_5, and 4.44 K_2O, or approximately 13 nitrogen, 1 phosphorus, and 8 potassium.

The above-mentioned investigations dealt with the level of nitrogen, potassium, and phosphorus found in the tissues of apple trees, but this level may not necessarily represent the ratio required for optimum growth. To study further the requirement of young apple trees for these three elements and to determine symptoms of moderate shortage, an experiment was set up in the greenhouse with trees grown in sand cultures. The studies herein reported describe the effect of different concentrations of nitrogen, potassium, and phosphorus upon tree growth and photosynthetic activity of the leaves.

[1] Received for publication August 12, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 115.

EXPERIMENTAL PROCEDURE

On February 5, 1937, seventy-two 1-year whips of York Imperial apples, grafted on French seedling piece roots, were carefully selected for uniformity in the nursery row and were brought to the greenhouse for potting. These trees were cut back to two buds above the graft union, and the roots were severely pruned in order to remove as much storage tissue as possible. Each tree, after being weighed, was planted in a 3-gallon glazed earthenware crock, in pure washed sand ground to medium fineness. A piece of glass tubing, fitted to a hole in the base of each crock, provided drainage. Distilled water was supplied to all trees until February 25. At that time the buds had developed into shoots approximately 2 inches long. Each tree was trained to a single shoot, and differential treatment was begun.

Full details of the nutrient solution used are given in table 1. Six trees received a complete nutrient solution (table 1, treatment A), containing 168 p. p. m. (parts per million) of nitrogen, 117 p. p. m. of potassium, and 93 p. p. m. of phosphorus, besides an adequate concentration of other elements generally recognized as essential for plant growth. This treatment represented the highest concentration of all three elements under consideration. The remaining 66 trees were divided into 3 series of 22 trees each, designated the nitrogen, potassium, and phosphorus series, respectively. Trees in each series were further divided into groups receiving treatments indicated in table 1. Four trees were included in each treatment with the exception of treatments F, M, and S, these having two trees each. In the nitrogen series the nitrogen content of the nutrient solution ranged from 0 to 60 p. p. m. Likewise, in the phosphorus and potassium series, the concentration of these elements ranged from 0 to 40 and from 0 to 60 p. p. m., respectively. In all series, nutrient elements other than the one varied were kept at full concentrations. The reactions of the freshly prepared nutrient solutions, as determined with the quinhydrone electrode, ranged from pH 5.9 to pH 6.9.

Two quarts of nutrient solution, consisting of the previous day's leaching made up to volume, was supplied daily to each tree. Complete change of nutrient solution and flushing of the sand were effected each week.

Temperature and humidity conditions in the greenhouse where these studies were conducted were subject to rather wide fluctuations. An attempt was made during the first 2 months of the experiment to hold the night temperature to 65° F., with the day temperature between 75° and 80°. During the summer months, alternate strips painted white on the greenhouse roof served to reduce the daytime temperature. During this period temperatures generally ranged between 80° and 105°.

Application of nutrients was discontinued on September 29, 1937, and the trees were harvested November 20. Measurements were made on tree diameter, length of terminal growth, and total linear growth including laterals when present. Total green weight of top was also recorded. Samples of bark, leaves, wood, and roots were analyzed for total organic nitrogen (*1*, *p. 20* [*22*]) and potassium. Samples for the last-named test were prepared for precipitation according to the method of Sideris (*11*). The method of precipitation was that of Wilcox (*15*).

TABLE 1.—*Composition of nutrient solutions* [1]

Series and treatment designation	Amount supplied in nutrient solution			Amount of stock solution per liter of nutrient solution[2]						
	N	P	K	1M $NaNo_3$	1M KH_2Po_4	1M $MgSO_4$	1M $CaCl_2$	1M $Ca(NO_3)_2$	1M NaH_2PO_4	1M KCl
Control (full nutrient solution):	*P. p. m.*	*P. p. m.*	*P. p. m.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
A	168	93	117	12.000	3.0	2.0	4.5			
Nitrogen series:										
B	60	93	117	4.260	3.0	2.0	4.5			
C	30	93	117	2.130	3.0	2.0	4.5			
D	15	93	117	1.080	3.0	2.0	4.5			
E	5	93	117	.360	3.0	2.0	4.5			
F	2	93	117	.144	3.0	2.0	4.5			
G	0	93	117	.000	3.0	2.0	4.5			
Potassium series:										
N	168	93	60			2.0		6.0	3.0	1.540
O	168	93	30			2.0		6.0	3.0	.770
P	168	93	10			2.0		6.0	3.0	.260
Q	168	93	4			2.0		6.0	3.0	.104
R	168	93	2			2.0		6.0	3.0	.052
S	168	93	0			2.0		6.0	3.0	.000
Phosphorus series:										
H	168	40	117			2.0		6.0	1.288	3.00
I	168	20	117			2.0		6.0	.644	3.00
J	168	10	117			2.0		6.0	.322	3.00
K	168	4	117			2.0		6.0	.129	3.00
L	168	2	117			2.0		6.0	.064	3.00
M	168	0	117			2.0		6.0	.000	3.00

[1] 0.5 p. p. m. of manganese and boron were supplied in all nutrient solutions. Iron was applied directly to the sand as ferric citrate.

[2] Nutrient solutions were made up in quantities of 18 to 20 liters; hence the quantities of stock solution per liter are reported to the third decimal.

In order to obtain a measure of the relative rate of growth and ultimate leaf size, daily leaf-area measurements were made on selected leaves from shortly after the time of unfolding until there was no further increase in area. For this purpose, two newly formed leaves on each tree were tagged during May and early June and daily measurements were made by tracing the outlines of the leaves on paper. A planimeter was used to determine the area.

Photosynthetic measurements were made according to the method of Heinicke and Hoffman (7) on individual leaves of typical plants.

EXPERIMENTAL RESULTS

NITROGEN SERIES

Typical trees of the nitrogen series photographed on August 13, are shown in figure 1. These pictures show clearly that, as the nitrogen level was increased in the nutrient solution, growth was correspondingly increased. Trees grown without nitrogen were characterized by rather short, slender stems bearing small yellowish-green leaves with reddish-colored petioles that formed relatively narrow angles with the stem. As the nitrogen content of the nutrient solution was increased, these characteristics became progressively less pronounced. Terminal bud formation on main shoots had not occurred on trees receiving treatments A, B, and C at the time of harvest (November 20), while terminal elongation of trees receiving treatment G had ceased as early as June 2, followed by similar cessation of growth in trees receiving treatments F, E, and D in the order mentioned.

Average growth measurements of trees in the nitrogen series are recorded in table 2. As determined by weight of tops, height of tree, growth of laterals, total linear growth, and diameter of stem, growth was significantly reduced with decreasing supplies of nitrogen in the

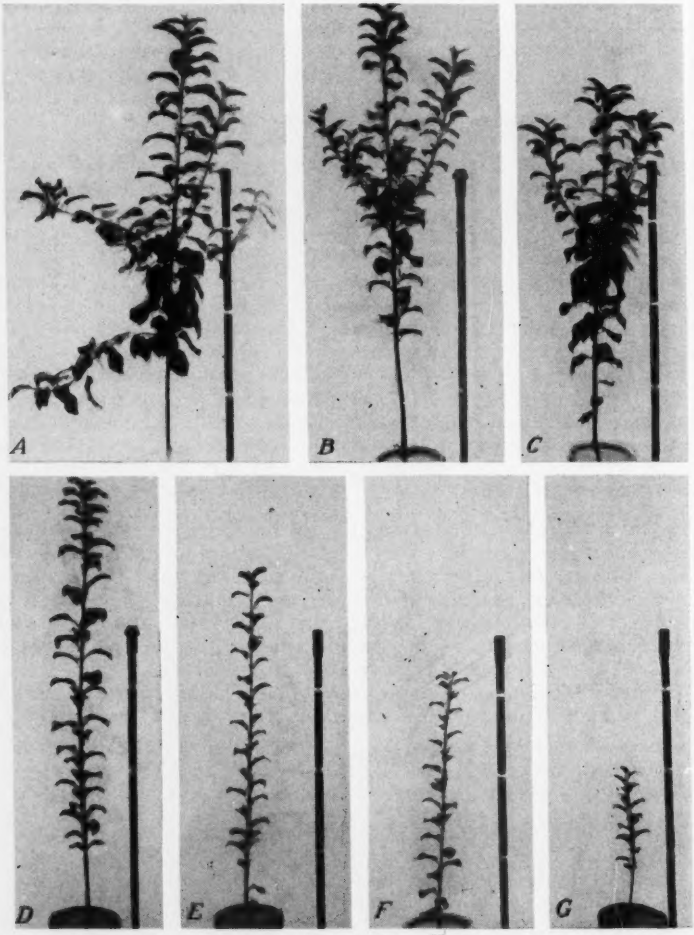

FIGURE 1.—Typical apple trees grown with various amounts of nitrogen in the nutrient solution: *A*, 168 p. p. m.; *B*, 60 p. p. m.; *C*, 30 p. p. m.; *D*, 15 p. p. m.; *E*, 5 p. p. m.; *F*, 2 p. p. m.; *G*, 0 p. p. m.

nutrient solution. The data clearly indicate that under the conditions of this experiment nitrogen was a limiting factor in growth when supplied in amounts less than 60 p. p. m. Since considerably less difference in growth, as measured by weight of tops, resulted from reducing nitrogen from 168 to 60 p. p. m. than from 60 to 30 p. p. m.,

it is probable that, with the apple, concentrations above 60 p. p. m. approach the luxury range for this element. Reduction below 60 p. p. m. of nitrogen in the nutrient solution reduced the amount of growth almost quantitatively. The greatest growth-rate increase, as measured by weight of tops, was obtained when the nitrogen level of the nutrient solution was advanced from 15 to 30 p. p. m.

TABLE 2.—*Effect of different concentrations of nitrogen, potassium, and phosphorus on growth of apple trees*

Series and treatment designation	Amount of N, P, or K in nutrient solution	Mean green weight of tops[1]	Mean height	Mean total length of laterals	Mean total linear growth	Mean diameter of trunk	CO_2 assimilated per 100 cm.[2 3]
Nitrogen series:	*P. p. m.*	*Gm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Mg.-hrs*
A[3]	168	341	181.3	408.4	589.7	1.67	23.5
B	60	313	175.3	348.1	523.4	1.66	22.7
C	30	232	178.9	172.3	351.2	1.42	15.8
D	15	106	157.4	.0	157.4	1.05	15.0
E	5	51	117.8	.0	117.8	.74	12.0
F	2	34	69.8	.0	69.8	.64	7.4
G	0	31	62.4	.0	62.4	.51	6.7
Potassium series:							
A[3]	117	341	181.3	408.4	589.7	1.67	23.6
N	60	314	184.1	297.7	481.8	1.66	19.8
O	30	227	170.2	144.8	315.0	1.39	19.0
P	10	197	175.6	78.4	254.0	1.33	17.8
Q	4	104	148.7	.0	148.7	1.06	17.0
R	2	84	137.6	.0	137.6	.98	14.1
S	0	51	111.4	.0	111.4	.78	13.6
Phosphorus series:							
A[3]	93	341	181.3	408.4	589.7	1.67	20.4
H	40	369	186.1	415.2	601.3	1.73	19.7
I	20	355	168.3	382.1	550.4	1.67	19.3
J	10	365	193.2	467.3	660.5	1.68	20.3
K	4	349	197.8	342.9	540.7	1.65	20.1
L	2	216	188.6	103.8	292.4	1.35	19.2
M	0	37	107.6	.0	107.6	.62	.2

[1] Differences between means required for significance according to Fisher's (t) method of anaylsis of variance are as follows: Nitrogen series, 52.9; phosphorus series, 60.5; potassium series, 54.8.
[2] Average of 24 or more determinations on 12 typical leaves of each treatment.
[3] Control (full nutrient solution).

The cumulative area of typical leaves of the nitrogen series is graphically illustrated in figure 2, which shows that the rate of growth, as well as the ultimate size, was markedly affected by the amount of nitrogen supplied in the nutrient solution. It is interesting to note that with all treatments approximately the same amount of time (12 to 15 days) was required for the leaves to reach maturity. It should be pointed out that the differences in leaf area represented in figure 2 are relative values for leaf growth at the time these measurements were made and are not representative of the entire leaf system of the trees. Difference between treatments in leaf size was considerably less earlier in the season because of the nitrogen reserve in the plant tissue prior to differential treatment. The no-nitrogen treatment is not included in figure 2 because terminal bud formation was under way when these measurements were started.

Not only was the total leaf area greater with increasing nitrogen supplied in the nutrient solution, but the apparent rate of photosynthesis per unit area was also greater (table 2). Carbon dioxide assimilation was measured simultaneously on representative leaves of trees receiving each treatment. The values in table 2 are averages of 24 or more determinations on 12 typical leaves for each nitrogen concentration. These data show that the rate of apparent photo-

synthesis was roughly proportional to the amount of nitrogen supplied, and that the average assimilation of leaves receiving 168 p. p. m. of nitrogen was 3.5 times that of leaves receiving no nitrogen. Significant differences were also obtained between treatments relatively high in nitrogen. Thus it may be seen that leaves receiving 60 p. p. m. assimilated 44 percent more carbon dioxide than leaves receiving 30 p. p. m.

Childers and Cowart (*3*) report similar results between assimilation rates of trees receiving full nutrient solution as compared with no-nitrogen treatments. Heinicke and Hoffman (*7*), comparing light- and dark-green leaves under field conditions, obtained three times the carbon dioxide assimilation in the case of the latter.

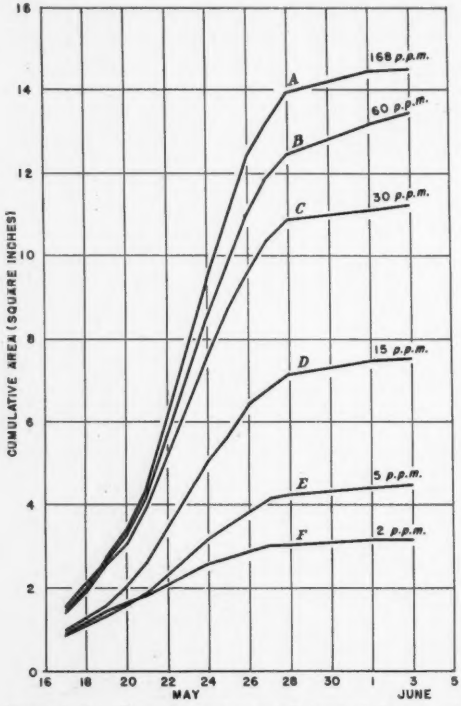

FIGURE 2.—Cumulative area of typical leaves of apple trees grown with various amounts of nitrogen in the nutrient solution.

POTASSIUM SERIES

Typical trees of the potassium series are shown in figure 3. It is to be seen here that a direct relationship existed between the amount of potassium supplied in the nutrient solution and growth response.

Trees receiving no potassium exhibited definite deficiency symptoms by early May. The lower leaves of these trees became light green in color, and later developed a rather pronounced chlorosis. This condition, in turn, was followed by typical scorching around the leaf margins. Later in the season the lower leaves of trees receiving the 2- and 4- p. p. m. treatments developed typical marginal scorching.

Growth measurements of trees in the potassium series are included in table 2. The trend of all measurements was strikingly similar to that in the nitrogen series. Growth, measured by weight of top, length of shoots, and diameter of stem, was roughly proportional to the amount of potassium supplied in the nutrient solution. The greatest increase occurred between 4 and 10 p. p. m. Even though visible symptoms of potassium deficiency did not occur on trees

receiving 10 p. p. m. or more, growth increased with increasing concentrations.

The effect of the potassium treatment on the growth of young leaves in early June is graphically illustrated in figure 4. The effect

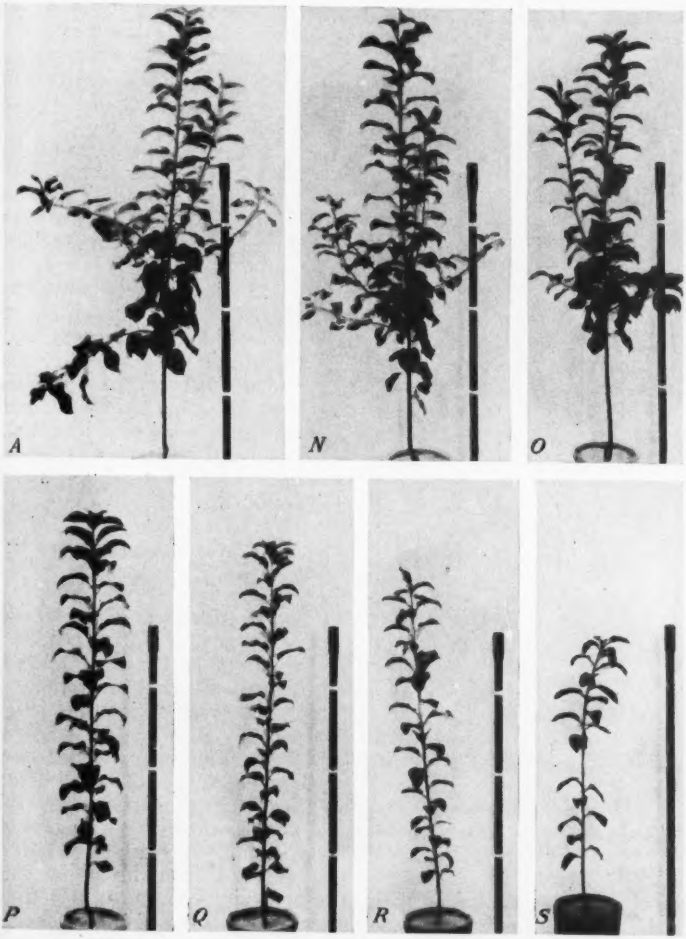

FIGURE 3.—Typical apple trees grown with various amounts of potassium in the nutrient solution: *A*, 117 p. p. m.; *N*, 60 p. p. m.; *O*, 30 p. p. m.; *P*, 10 p. p. m.; *Q*, 4 p. p. m.; *R*, 2 p. p. m.; *S*, 0 p. p. m.

is less pronounced than in the nitrogen series, but the trend is the same. However, it will be noted that, at a concentration of 30 p. p. m. or less, the rate of growth and ultimate size of leaf are considerably greater than in leaves receiving the same concentrations of nitrogen.

PHOSPHORUS SERIES

Decreasing amounts of phosphorus supplied in the nutrient solution had no effect on tree growth (fig. 5) until a concentration of less than 4 p. p. m. was reached. At 2 p. p. m. of phosphorus, the trees did not exhibit any of the usual deficiency symptoms; however, the growth measurements show a significant downward trend as compared with those of trees receiving higher concentrations of the element (table 2 and fig. 6). The no-phosphorus trees were typically short and slender, with small dark-green leaves of a purplish cast. Symptoms closely resembled those of the no-nitrogen trees except for the color of foliage.

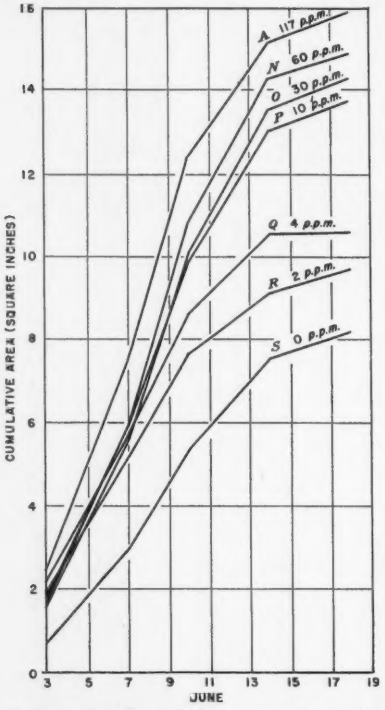

FIGURE 4.—Cumulative area of typical leaves of apple trees grown with various amounts of potassium in the nutrient solution.

These results indicate that the phosphorus requirement of apple trees is very low as compared with the requirements for nitrogen and potassium. Because of the numerous failures to obtain response to phosphorus under field conditions, it would seem that soils generally have available a sufficient supply of this element to meet the relatively small needs of the apple tree.

NITROGEN AND POTASSIUM ANALYSES

The nitrogen and potassium analyses of plant tissue in the nitrogen and potassium series are summarized in table 3. In the nitrogen series the nitrogen content of both leaf and bark tissue is directly related to the concentration of this element in the nutrient solution. With wood and root tissue, some discrepancies are apparent but the trend is roughly the same. Likewise, in the potassium series the potassium content of the tissues generally increases with increasing concentrations of this element in the nutrient solution and in turn is directly associated with growth response.

The data in table 3 show clearly that the potassium content of leaves and bark in the nitrogen series and the nitrogen content of these tissues in the potassium series are independent of differential treatment. Thus it may be seen that the potassium content is virtually constant throughout the nitrogen series. Similarly, the nitrogen

content of the leaves and bark in the potassium series is relatively constant, with the potassium ranging from 0 to 117 p. p. m. It would therefore seem that within the limits of these concentrations there does not exist an unfavorable balance between nitrogen and potassium.

FIGURE 5.—Typical apple trees grown with various amounts of phosphorus in the nutrient solution: *A*, 93 p. p. m.; *H*, 40 p. p. m.; *I*, 20 p. p. m.; *J*, 10 p. p. m.; *K*, 4 p. p. m.; *L*, 2 p. p. m.; *M*, 0 p. p. m.

LEAF ASSIMILATION AS AFFECTED BY INCREASING THE NITROGEN CONTENT OF THE NUTRIENT SOLUTION

On August 23, after terminal growth had ceased at the lower nitrogen levels, two trees from each of the 2-, 5-, and 15-p. p. m. treatments of the nitrogen series (fig. 7) were selected for determining the effect of increasing the nitrogen content of the nutrient solution on carbon dioxide assimilation. Preliminary measurements (table 4) were made simultaneously on selected leaves of each tree to determine the relative rate of carbon dioxide assimilation. On August 28, one tree of each

pair (trees 4, 2, and 6) received a complete nutrient solution containing 168 p. p. m. of nitrogen. The other tree of each pair (trees 3, 1, and 5) continued to receive the usual treatment. From August 31 to September 29, three series of determinations were made which are summarized in table 4. A typical tree, receiving a complete nutrient solution (168 p. p. m. of nitrogen) throughout the season, was included in this experiment to serve as a basis for comparison.

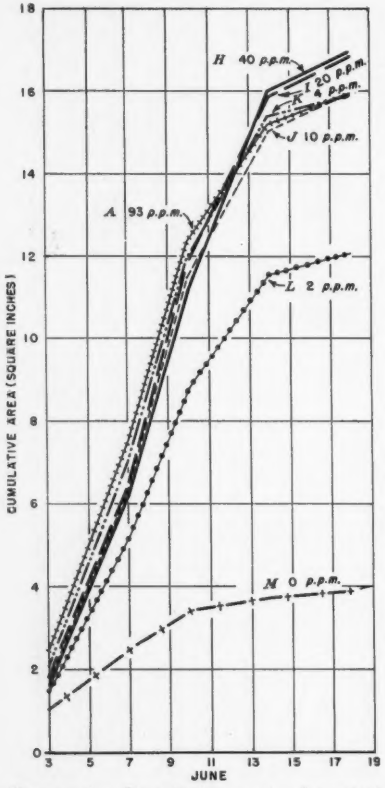

FIGURE 6.—Cumulative area of typical leaves of apple trees grown with various amounts of phosphorus in the nutrient solution.

The foliage of all trees receiving full nutrient solution on August 28 became noticeably greener within a few days after the application was made. From August 31 to September 8 there was a definite increase in rate of photosynthesis of these trees as compared with their companion trees, which continued to receive the usual concentration. During this period, tree 6, which formerly received 2 p. p. m. of nitrogen, more than doubled its rate of assimilation as compared with its control tree (table 4). The data in table 4 clearly show that in all cases the rate of assimilation of the foliage was increased by increasing the nitrogen content of the nutrient solution. Approximately 1 month after additional nitrogen was added, the photosynthetic rate of tree 6 had increased more than threefold as compared with tree 5. Tree 2 had almost doubled its rate, and tree 4 had increased by two-thirds as compared with its companion tree. Heinicke (*5*) reports similar increases in assimilation as a result of late summer applications of nitrogen under field conditions. Besides a large increase in rate of assimilation the foliage of trees receiving the full nutrient solution after August 28 was still active several weeks after their companion trees were completely defoliated (fig. 8).

The increase of nitrogen intake as a result of increasing the supply of nitrogen in the nutrient solution is clearly indicated in table 4. On September 28, the nitrogen content of the foliage of trees 2, 4, and 6 had equaled or exceeded that of tree 7, which had received a full nutrient solution throughout the season. It is interesting to note (table 4) that the rate of increase (as compared with their com-

panion trees) in the nitrogen content of the leaves of these trees was closely proportional to the percentage increase in assimilation. Tree 2 had a nitrogen content of 1.9 times that of its companion tree (table 4) and it also assimilated 1.9 times as much carbon dioxide (table 4, September 20 to 29) as its companion tree. Trees 4 and 6 show similar relationships between nitrogen content of foliage and rate of assimilation as compared to their respective companion trees.

TABLE 3.—*Summary of nitrogen and potassium analyses*

Series and treatment designation	N contained in nutrient solution	K contained in nutrient solution	N content of—				K content of—	
			Leaves, Oct. 25	Bark, Nov. 20	Roots, Nov. 20	Wood, Nov. 20	Leaves, Oct. 25 [1]	Bark, Nov. 20
Nitrogen series:	*P. p. m.*	*P. p. m.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A [2]	168	117	1.856	1.488	1.256	0.555	2.31	1.049
B	60	117	1.702	1.179	.636	.367	2.35	1.053
C	30	117	1.392	1.008	1.072	.346	2.29	1.122
D	15	117	1.229	.939	1.107	.369	2.35	1.144
E	5	117	1.148	.801	.763	.315	1.93	.861
F	2	117	.940	.776	.948	.310	2.00	1.018
G	0	117	-------	.573	.357	.182	-------	-------
Potassium series:								
A [2]	168	117	1.856	1.488	-------	-------	2.31	1.049
N	168	60	1.839	1.416	-------	-------	1.95	.953
O	168	30	1.819	1.581	-------	-------	1.69	.815
P	168	10	1.814	1.401	-------	-------	.83	.682
Q	168	4	1.786	1.691	-------	-------	.68	.740
R	168	2	1.649	1.787	-------	-------	.66	.712
S	168	0	1.620	2.084	-------	-------	.33	.639

[1] Differences between leaf values of different treatments required for significance, according to Fisher's (4) method of analysis of variance, are as follows: Nitrogen series, 0.124 for nitrogen and 0.322 for potassium; potassium series, 0.218 for nitrogen and 0.228 for potassium.
[2] Control (full nutrient solution).

TABLE 4.—*Effect of nitrogen increase in nutrient solution on nitrogen content and CO_2 assimilation of apple leaves*

Tree No.	Nitrogen in nutrient solution prior to Aug. 28	Nitrogen in nutrient solution after Aug. 28	CO_2 assimilated per 100 cm.^{2} Aug. 23–28 [1]	CO_2 assimilated [2]			Nitrogen content [3] of—			
				Aug. 31–Sept. 8	Sept. 9–18	Sept. 20–29	Leaves, Sept. 28	Bark, Nov. 20	Wood, Nov. 20	Roots, Nov. 20
	P. p. m.	*P. p. m.*	*Mg.-hrs.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
7	168	168	16.0	121.7	122.0	145.0	2.10	1.50	0.57	0.91
3	15	15	11.4	126.3	122.4	99.7	1.37	.77	.28	1.19
4	15	168	9.5	145.7	157.2	165.3	2.07	2.19	.85	1.54
1	5	5	9.3	116.3	90.0	105.0	1.15	.81	.34	.77
2	5	168	7.0	146.0	172.0	199.7	2.18	1.65	.96	1.72
5	2	2	4.2	81.0	72.2	68.7	.94	.70	.23	.91
6	2	168	4.3	169.3	272.8	230.0	2.58	1.88	.89	1.54

[1] Each value is an average of 6 determinations made on 2 leaves of each tree. The same 2 leaves on each tree were used throughout the test.
[2] Expressed as percentage of CO_2 assimilated from Aug. 23 to 28.
[3] Percentage of dry weight.

Data on nitrogen content of bark, wood, and roots of trees included in this experiment are shown in table 4 and clearly indicate large increases in nitrogen content of these tissues as a result of nitrogen increase in the nutrient solution. Tree 7 was still making active terminal growth at the time these samples were taken, which perhaps accounts for the relatively low nitrogen content of the wood and roots of this tree.

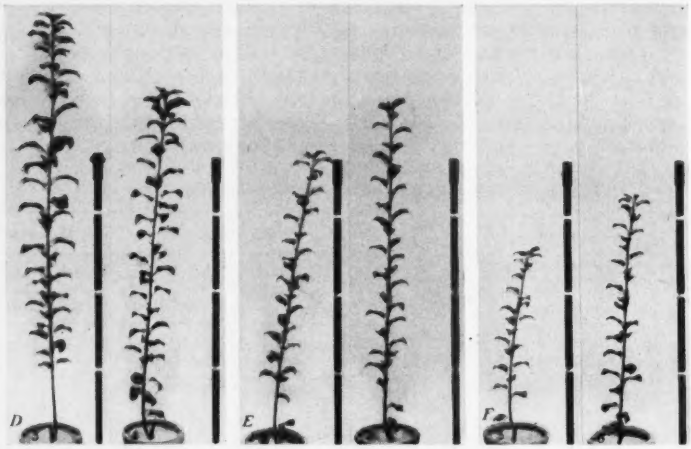

FIGURE 7.—Apple trees grown with various amounts of nitrogen in the nutrient solution; treatment D, 15 p. p. m. (trees 3 and 4); treatment E, 5 p. p. m. (trees 1 and 2); treatment F, 2 p. p. m. (trees 5 and 6). Photographed August 23.

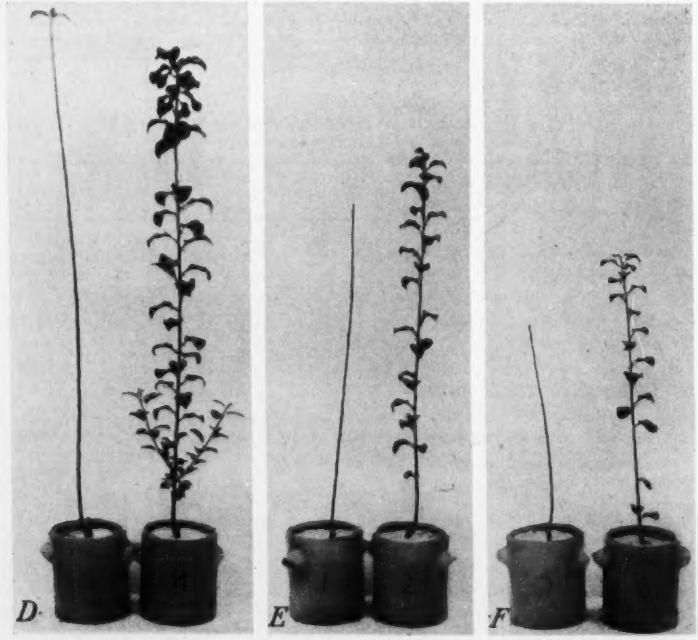

FIGURE 8.—Same trees as shown in figure 7. Trees 4, 2, and 6 received complete nutrient solution beginning August 28. Photographed September 29.

DISCUSSION

It should be emphasized that the concentration of nutrients used in these tests represents the amounts in the water added to the sand. Since many orchard soils hold from 20 to 30 percent of water, it seems probable that 15 p. p. m. based on a soil analysis would roughly approximate 60 p. p. m. in the water as used here.

Under the conditions of this test, significant differences in growth in the nitrogen series were obtained when the nitrogen was reduced from 60 to 30 p. p. m. A slight increase in growth occurred between 60 and 168 p. p. m., but this was less than was required for significance.

In the potassium series, also, significant differences in green weight of top occurred when the potassium content was reduced from 60 to 30 p. p. m. This would indicate that approximately similar concentrations of available potassium and available nitrogen are required for optimum growth of young apple trees.

It is interesting to note the effect of nutrient concentration upon lateral branching. In the nitrogen series the development of laterals did not occur below the 30 p. p. m. treatment; in the potassium series there was no branching below 10 p. p. m.

The wide variation in the potassium content of the leaves of trees receiving different amounts of potash is particularly interesting. With 30 p. p. m. of potassium in the nutrient solution, leaves showed 1.69 percent of potassium on a dry-weight basis. With this concentration in the leaves, growth was significantly reduced when all other nutrients were available in ample quantities. The analyses of leaves from the 4-p. p. m. treatment showed a reduction to 0.68 percent of potassium on a dry-weight basis. This wide fluctuation in the content of the leaves from trees furnished with various amounts of this element suggests the value of determining the potassium content of the leaves as a measure of potassium availability under field conditions. In these tests, trees responded to additional potassium when leaf analyses showed 1.69 percent of potassium present. It is probable, of course, that had the nitrogen also been reduced on these trees, no response from additional potash would have been observed. Under orchard conditions nitrogen is usually a limiting factor in obtaining maximum growth, and response to potash applications could not be expected until the full nitrogen requirement had been supplied, unless the potash availability was extremely low.

The fact that definite symptoms of potassium deficiency were not observed until the potash content of the nutrient solution was reduced to less than 10 p. p. m. indicates that response to potash applications may be expected under some conditions where no visible symptoms of deficiency can be observed.

In the phosphorus series, significant reduction in growth of trees did not occur until the phosphorus content of the nutrient solution was reduced to less than 4 p. p. m. In the present work, the phosphorus concentration required for optimum growth was approximately only one-fifteenth of the concentration required for nitrogen and potassium. Thus this study substantiates results of earlier workers in indicating that the phosphorus requirement of apple trees is very low as compared with nitrogen and potassium.

The fact that the potassium concentration in the nitrogen series did not increase as growth of trees was reduced by lack of nitrogen

indicates that marked luxury storage of potassium under these conditions did not occur. Similarly, the fact that the nitrogen content of the trees in the potassium series where growth was reduced by lack of potassium did not increase significantly indicates that luxury absorption of nitrogen did not occur under these conditions. Neither the absorption of nitrogen in the potassium series nor the absorption of potassium in the nitrogen series was appreciably affected by varying the concentration of the other element.

The results of photosynthetic measurements on the trees in the nitrogen series substantiate results of earlier work in showing a marked decrease in photosynthetic activity of the leaves under reduced nitrogen conditions. The photosynthetic measurements on the trees in the potassium series indicate that the effects of decreased potassium on leaf efficiency are similar to but less marked than the effects of decreased nitrogen.

SUMMARY

One-year York Imperial apple trees were grown in the greenhouse in sand cultures in a study of the effects of various concentrations of nitrogen, potassium, and phosphorus upon growth response and photosynthetic activity of leaves. The following concentrations were used: Nitrogen, 0, 2, 5, 15, 30, 60, and 168 p. p. m.; potassium, 0, 2, 4, 10, 30, 60, and 117 p. p. m.; and phosphorus, 0, 2, 4, 10, 20, 40, and 93 p. p. m. In all series, nutrient elements other than the one studied were kept at full concentration.

Trees grown with nitrogen at 60 p. p. m. made somewhat less growth, as measured by total linear growth and total fresh weight, than trees grown with 168 p. p. m., though the differences were not statistically significant. Concentrations of nitrogen below 60 p. p. m. reduced the amount of growth almost quantitatively, the largest difference in growth resulting at concentrations between 15 and 30 p. p. m. Potassium at 60 p. p. m. gave less growth than at 117 p. p. m., and less growth with each decreasing concentration. The greatest difference in growth occurred between 4 and 10 p. p. m. Growth with 2, 4, 10, and 30 p. p. m. was not decreased as much as with corresponding concentrations of nitrogen. Definite deficiency symptoms in the potassium series did not occur on trees receiving 10 p. p. m. or more, although growth increased with increasing concentrations until more than 60 p. p. m. were supplied. In the phosphorus series, growth was approximately uniform in all treatments receiving 4 p. p. m. or more of this element. Only when phosphorus was completely lacking did visible symptoms of deficiency occur.

The nitrogen and potassium content of the plant tissues, particularly of the leaves and bark, increased with increasing concentrations of these elements in the nutrient solution. The potassium content of leaves and bark in the nitrogen series and the nitrogen content of these tissues in the potassium series were found to be independent of differential treatment.

The rate of photosynthesis per unit of leaf area was markedly reduced in the nitrogen series with decreasing amounts of this element in the nutrient solution. A similar but less marked decrease occurred in the potassium series. A significant decrease in assimilation occurred in the phosphorus series only with the complete absence of this element.

Trees grown at a low nitrogen level until the terminal bud had formed were supplied with a high nitrogen solution in late August. Carbon dioxide assimilated per unit area of leaf was measured periodically prior to and immediately following the application of the higher nitrogen solution. Leaves became noticeably greener within a few days after additional nitrogen was supplied. Carbon dioxide assimilation per unit area of leaf significantly increased during this period, and 21 days after application of increased nitrogen it had doubled in most cases. The increase in nitrogen content of the leaves was closely correlated with the increase in assimilation.

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INHERITANCE IN THE CUCUMBER[1]

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INTRODUCTION

In connection with breeding work conducted during the past few years, studies have been made on the inheritance of several characters in the cucumber *Cucumis sativus L.* The results of a portion of these studies are reported in this paper.

MATERIALS AND METHODS

The data herein presented were obtained from a study of the F_1 and F_2 progenies of a cross made in 1934 between two inbred cucumber parents that differed in certain characters as shown in table 1.

TABLE 1.—*Contrasting characters of two inbred cucumber parents*

Characters	Parents	
	Male	Female
Growth habit	Determinate	Indeterminate.
Height of plant	Short	Tall.
Spine color	Black	White.
Spine size	Fine	Coarse.
Spine frequency	Numerous	Few.
Mature fruit color	Red	Cream.
Mature fruit netting	Heavy	None or very slight
Number of mature fruits per plant	Few	Many.
Total weight of mature fruit per plant	Small	Large.
Average weight (size) of mature fruit per plant	do	Do.
Total number of fruit per plant (mature + green)	Few	Many.
Average weight of each fruit per plant (mature + green)	Small	Large.
Days after planting to first male flower	Few	Many.
Days after planting to first female flower	Many	Few.
Number of female flowers per plant	Few	Many.
Number of laterals per plant	do	Do.
Average length of lateral per plant	Short	Long.
Total length of all laterals per plant	Small	Large.

Of the characters mentioned in table 1, determinate growth habit requires, perhaps, further explanation. In a strain of cucumbers received from the Union of Soviet Socialist Republics in 1928, a plant appeared which was determinate in growth habit. In this plant and succeeding inbred progenies derived from it, the apical growth terminated in a cluster of male or female flowers, the laterals were few and very short, and plant height ranged from 6 to 12 inches depending upon environmental conditions (fig. 1).

In the F_1 and F_2 populations, grown in the greenhouse, all female flowers produced were hand-pollinated and the fruits were permitted to remain on the plant until the plant appeared to have reached its

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production capacity and started to die, when all fruits and the plant itself were harvested and final measurements taken. F_3 lines representing each F_2 plant were grown in the field and the plants treated in the same manner as the F_2 plants except that open pollination was allowed to take place.

FIGURE 1.—Dwarf determinate cucumber parent in foreground; indeterminate parent in background

GENETIC ANALYSIS

The ratios obtained for the various characters in the F_2 population, which consisted of 151 plants in the plant height study and 156 plants in all others, are given in table 2.

TABLE 2.—*Differential characters of the two parents, and appearance of the F_1 and of the segregates obtained in the F_2 of a cucumber cross*

Characters	Parents		F_1	F_2 segregates [1]	χ^2 test for goodness of fit; P lies between—[2]
	Male	Female			
Growth habit	Determinate	Indeterminate	Determinate	36 determinate [3] 81 segregating [3] 39 indeterminate [3]	0.80 and 0.90
Plant height	Short	Tall	Intermediate	31 tall [3] 79 intermediate [3] 41 short [3]	.30 and .50
Spine color	Black	White	Black or brown	45 black [3] 69 segregating [3] 42 white [3]	.30 and .50
Spine size	Fine	Coarse	Intermediate	35 coarse [3] 82 segregating [3] 39 fine [3]	.70 and .80
Spine frequency	Numerous	Few	Intermediate	35 few [3] 82 segregating [3] 39 numerous [3]	.70 and .80
Mature fruit color	Red	Cream	Red	85 red [4] 29 orange [4] 32 yellow [4] 10 cream [4]	.90 and .95
Mature fruit netting	Heavy	None or very slight.	Heavy	40 heavy [3] 77 segregating [3] 39 none or very slight [3]	.98 and .99

[1] Classified according to the behavior of F_3 lines.
[2] Involving a 1 : 2 : 1 ratio.
[3] Fisher's table of χ^2 (5).[5]
[4] Involving a 9 : 3 : 3 : 1 ratio.
[5] Italic numbers in parentheses refer to Literature Cited, p. 127.

GROWTH HABIT

From table 2 it can be seen that the F_1 progeny from the cross between determinate (II) and indeterminate (*ii*) types was determinate. In the F_2, 117 determinate and 39 indeterminate (*ii*) plants were obtained. Of the 117 determinate plants, 36 proved to be homozygous determinate (*II*) and 81 heterozygous (*Ii*) in the F_3 generation. Similar results would be expected if the difference between the parents was due to a single factor.

A similar type of determinate growth in the tomato has been reported by Yeager (*8*) and Currence (*2*) as differing by a single factor from the indeterminate habit. Currence found that the determinate habit behaved as a recessive in the tomato, which is in contrast to the behavior of this character in the cucumber.

PLANT HEIGHT

The difference between the two parents in plant height appears to be dependent on a single factor in this cross. As shown in table 2, the F_1 was intermediate. In the F_2, 31 tall (*TT*), 79 intermediate (*Tt*), and 41 short (*tt*) were obtained.

SPINE COLOR

As shown in table 2, the spines on the fruits of F_1 plants were classified as black or brown. In the F_2, black or brown spines were observed on 114 plants and white spines (*bb*) on 42. In the F_3, 45 of the 114 plants having black or brown spines proved to be homozygous (*BB*) for black spines and 69 heterozygous (*Bb*) for spine color. The results suggest that a single factor difference existed between the parents for spine color. These results agree with those reported by Wellington (*7*), Strong (*4*), and Tkachenko (*5*).

SPINE SIZE

As indicated in table 2, spine size was intermediate in the F_1. In the F_2, coarse or intermediate spines were observed on 117 plants and fine spines (*ff*) on 39 plants. In the F_3, 82 of the 117 plants having coarse or intermediate spines segregated for spine size and 35 proved to be homozygous (*FF*) for coarse spines. This suggests that a single factor difference existed between the parents for spine size. These results agree with those reported by Wellington (*7*). Strong (*4*), from observations made on the F_2 progeny of a cucumber cross, Vickery $\times$ Everyday, suggested that spine size probably was inherited on a multiple factor basis.

SPINE FREQUENCY

In the F_1 of this cross, frequency or number of spines appeared to be intermediate. As shown in table 2, the F_2 consisted of 117 plants having few or an intermediate number of spines and 39 plants having numerous (*ss*) spines. In the F_3, 82 of the 117 plants having few or an intermediate number of spines segregated and 35 proved homozygous (*SS*) for few spines. It is suggested that a single factor difference existed between the parents for spine frequency, which agrees with the results obtained by Wellington (*7*), Strong (*4*), and Tkachenko (*5*).

MATURE FRUIT COLOR

In studying the inheritance of mature fruit color, the color was found to be red in the F_1. As shown in table 2, the ratio obtained in the F_2 very closely approaches the theoretical 9:3:3:1 ratio that would be expected on the basis of a two-factor difference between the parents. The four classes obtained were red (*RC*), orange (*Rc*), yellow (*rC*), and cream (*rc*). Homozygous F_3 lines were obtained for all these colors. The data indicate that the red character is due to both factors being present in a dominant (*RRCC*) condition; the cream to both factors being present in a homozygous recessive condition (*rrcc*); and the orange and yellow to one factor being present in the homozygous recessive and the other factor in either the homozygous dominant or heterozygous condition. In the greenhouse, the yellow is a bright canary yellow, readily distinguished from the cream. In the field, the yellow closely approaches the cream and is often difficult to distinguish from it.

MATURE FRUIT NETTING

From table 2 it can be seen that fruit of the F_1 plants obtained from the cross between the heavy-netted (*HH*) and no-netted (*hh*) types was heavily netted. In the F_2 the fruits of 117 plants showed heavy or intermediate netting and 39 slight or no netting (*hh*). In the F_3, 40 plants of the 117 showing heavy or intermediate netting proved to be homozygous for heavy netting (*HH*) and 77 segregated for this character. It is suggested that a single factor difference existed between the two parents for mature fruit netting, which agrees with the results of Tkachenko *(5)*.

INTERACTION OF GROWTH HABIT AND PLANT HEIGHT

The determinate growth and the plant height genes appear to exert considerable influence on each other as shown to some extent in table 3, where the phenotypic classes obtained in the interaction of the two factors in the F_2, together with the height range of the phenotypic classes, is given.

TABLE 3.—*Height range in each of four phenotypic classes obtained in the F_2 progeny of a cross between tall-indeterminate and short-determinate cucumber lines*

Phenotypic classes	Segregation obtained in the F_2	
	Observed	Height class
	Number	*Inches*
Determinate-tall (*IT*)	80	19–110
Determinate-short (*It*)	32	6–19
Indeterminate-tall (*iT*)	30	71–136
Indeterminate-short (*it*)	9	32–71

The segregation given in table 3 agrees with the theoretically expected 9:3:3:1 dihybrid ratio. Of interest is the effect of the interaction of the genes *I*-determinate and *T*-tall on height of plant. When the gene *I* for determinate growth habit is present together with the gene *t* for short plant height, the plants are very short. When *I* is present with the gene *T* for tall plant height, the plants are consider-

ably taller. When the indeterminate growth gene *i* is present, both the *T* and *t* plants are considerably taller than in the comparable classes of *I* plants. These data indicate that the *I* factor for determinate growth, when present, tends to decrease the height of the plant. The *I* gene exhibits partial dominance in this respect. The *II* plants are shorter than the *Ii* plants. Attention has been called to the intermediate condition of the *Tt* plants as compared to the parents.

TESTS FOR INDEPENDENT ASSORTMENT

Several characters just discussed were tested in all possible combinations for independent assortment by the use of the χ^2 test for independence. The results of these tests between the independently assorted characters and between characters where the data, although indicating association, do not appear to be sufficient to warrant definite conclusions are presented in table 4. The ratios were determined on 156 F_2 plants checked by F_3 lines from each of these plants.

TABLE 4.—*Tests for independent assortment of seven factors responsible for six characters under observation*

Parents	F_2 plants in indicated phenotypic classes	χ^2 test for independent assortment; P[1] lies between—
	Number	
Coarse, white spines × fine, black spines (*FFbb*×*ffBB*).	*BF*, 85; *Bf*, 29; *bF*, 32; *bf*, 10	0.80 and 0.90
Few, white spines × numerous, black spines (*SSbb*×*ssBB*).	*BS*, 85; *Bs*, 29; *bS*, 32; *bs*, 10	.80 and .90
White spines, indeterminate × black spines determinate (*bbii*×*BBII*).	*BI*, 86; *Bi*, 28; *bI*, 31; *bi*, 11	.80 and .90
Coarse spines, indeterminate × fine spines, determinate (*FFii*×*ffII*).	*FI*, 86; *Fi*, 31; *fI*, 31; *fi*, 8	.30 and .50
Few spines, indeterminate × numerous spines, determinate (*SSii*×*ssII*).	*SI*, 86; *Si*, 31; *sI*, 31; *si*, 8	.30 and .50
No netting, coarse spines × heavy netting, fine spines (*hhFF*×*HHff*).	*HF*, 86; *hF*, 31; *Hf*, 31; *hf*, 8	.30 and .50
No netting, few spines × heavy netting, numerous spines (*hhSS*×*HHss*).	*HS*, 86; *hS*, 31; *Hs*, 31; *hs*, 8	.30 and .50
No netting, indeterminate × heavy netting, determinate (*hhii*×*HHII*).	*HI*, 89; *hI*, 28; *Hi*, 28; *hi*, 11	.50 and .70
Coarse spines, cream fruit × fine spines, red fruit (*FFrrcc*×*ffRRCC*).	*FRC*, 58; *fRC*, 28; *FRc*, 27; *fRc*, 1; *FrC*, 22; *frC*, 10; *Frc*, 10; *frc*, 0.	.01
Few spines, cream fruit × numerous spines, red fruit (*SSrrcc*×*ssRRCC*).	*SRC*, 58; *sRC*, 28; *SRc*, 27; *sRc*, 1; *SrC*, 22; *srC*, 10; *Src*, 10; *src*, 0.	.01
Inderterminate, cream fruit × determinate, red fruit (*iirrcc*×*IIRRCC*).	*IRC*, 64; *iRC*, 22; *IRc*, 22; *iRc*, 6; *IrC*, 25; *irC*, 7; *Irc*, 6; *irc*, 4.	.50 and .70

[1] Fisher's table of χ^2 (*3*).

INDEPENDENTLY ASSORTED CHARACTERS

In table 4, *P* values within the range 0.1 to 0.9 suggest no significant association between the characters for which they were obtained (*3*). Therefore, the following characters appear to be inherited independently of each other: Spine color and spine size; spine color and spine frequency; spine color and growth habit; spine size and growth habit; spine size and mature fruit netting; spine frequency and growth habit; spine frequency and mature fruit netting; growth habit and mature fruit color; and growth habit and mature fruit netting.

SPINE SIZE AND FREQUENCY AND MATURE FRUIT COLOR

Both spine size and frequency appeared to be associated with mature fruit color. A χ^2 of 13.48 and a *P* value of less than 0.01 was

obtained. Considering the number of classes involved, however, the population is so small that a small error in classification could easily change the χ^2 enough to indicate independence.

CHARACTERS APPEARING TO SHOW ASSOCIATION

In these tests for independence, the following characters appeared to show complete association: Spine size and spine frequency, spine color and fruit color, fruit netting and spine color, and fruit netting and fruit color. χ^2 values of from 141.2 to 156.0 were obtained and the P values in all cases are less than 0.01. These characters will be discussed more fully in the following paragraphs.

Spine Size and Frequency

Coarse spines appear to be associated with few spines and fine spines with numerous spines. When the female parent having coarse, few spines (*FFSS*) on the fruits was crossed with the male parent having fine numerous (*ffss*) spines, a ratio of 117(*FS*):0(*Fs*):0(*fS*):39(*fs*) was obtained in the F_2 segregation. Since no plants were obtained in the coarse, numerous or fine, few spines classes, complete association is indicated between spine size and spine frequency in this cross.

Spine Color and Mature Fruit Color

Black spines appear to be associated with red and orange fruit color and white spines with yellow and cream fruit color. When the female parent having cream fruit with white spines (*ccrrbb*) was crossed with the male parent having red fruit with black spines (*CCRRBB*), a ratio of 86(*CRB*):0(*CRb*):28(*cRB*):0(*cRb*):0(*CrB*):32(*Crb*):0(*crB*):10(*crb*) was obtained in the F_2 segregation. Since no plants were obtained having red fruit and white spines, orange fruit and white spines, yellow fruit and black spines, or cream fruit and black spines, complete association is indicated between spine color and one of the two genes responsible for mature fruit color. Tkachenko (*5*) also concludes that white spines and white (colorless) mature fruit color are completely linked. Cochran (*1*) found no linkage between fruit color and spine color in an investigation in which a variety having green fruit and white spines was crossed with a variety having white fruit and black spines.

Spine Color and Mature Fruit Netting

Heavy netting appears to be closely associated with black spines and no netting with white spines. When the female parent having no netting and white spines (*hhbb*) was crossed with the male parent having heavy netting and black spines (*HHBB*), a ratio of 114(*HB*):0(*hB*):3(*Hb*):39(*hb*) was obtained in the F_2 segregation. No plants were obtained having slight or no netting and black spines and only three plants having heavy netting and white spines. Tkachenko (*5*) found that complete linkage exists between white spines and smooth fruit surface. These results appear to show the same thing; the small deviation that occurs in the white spine—heavy netting class possibly being due to errors in classification.

Mature Fruit Netting and Mature Fruit Color

Heavy netting also appears to be closely associated with red and orange fruit color and no netting with yellow and cream fruit color. When the female parent having no netting and cream fruit (*hhrrcc*) was crossed with the male parent having heavy netting and red fruit (*HHRRCC*), a ratio of

86(*HRC*):0(*hRC*):28(*HRc*):0(*hRc*):3(*HrC*):29(*hrC*):0(*Hrc*):10(*hrc*)

was obtained in the F_2 segregation. No plants were obtained having no netting and red fruit, no netting and orange fruit, heavy netting and cream fruit, and only three plants having heavy netting and yellow fruit. These results appear to indicate complete or nearly complete association between mature fruit netting and color and agree partly with the results of Tkachenko (*5*), who found complete linkage between white (colorless) mature fruit and smooth fruit surface. The small deviation that occurs in the heavy netting-yellow mature fruit class may be due, possibly, to errors in classification.

ASSOCIATION OF QUALITATIVE AND QUANTITATIVE CHARACTERS

In addition to the characters discussed previously a number of others were included in the study. The degree of association between these additional characters and those previously discussed is indicated in table 5, in which *t* values obtained in a comparison of the means of the phenotypic classes are presented. Observations were made on 149 to 156 individuals.

TABLE 5.—*Association between various characters in the F_2 progeny of a cucumber cross as indicated by the* t *values obtained in a comparison of the means of the phenotypic classes*

Characters	Plant height (inches)	Number of mature fruits	Total weight of mature fruit (grams)	Weight (size) per mature fruit	Total number of fruits (mature + green)	Total weight of fruit (mature + green)	Weight per fruit (mature + green)	Days to first male flower	Days to first female flower	Number of female flowers	Number of laterals	Length per lateral	Total length of laterals
Spine color	0.06	1.24	0.30	[1] 2.39	1.07	0.60	[2] 2.91	1.29	1.05	0.25	1.31	1.54	1.20
Spine size	1.15	.03	1.18	[2] 3.34	.90	1.80	[1] 2.59	1.02	1.58	.25	2.34	.67	.50
Spine frequency	1.51	.03	1.18	[2] 3.34	.90	1.80	[1] 2.59	1.02	1.58	.25	2.34	.67	.50
Growth habit	[2] 10.22	[2] 18.63	[2] 7.26	[2] 4.46	[2] 7.29	[2] 7.99	[2] 4.49	.25	[1] 2.35	[2] 5.79	[2] 4.64	[2] 4.81	[2] 7.05
Mature fruit color:													
Red	.69	1.19	.25	1.56	.91	.04	1.53	.06	1.50	1.09	.68	1.09	[2] 3.71
Orange	1.50	1.49	.93	.64	.79	.83	.08	.91	.91	[2] 2.77	.17	.67	1.43
Yellow	.46	1.29	.42	.85	1.28	.30	1.08	.73	.65	.22	1.26	1.40	1.40
White	1.20	.33	1.24	[2] 2.76	.47	1.35	[2] 2.99	.99	.44	.46	.12	.24	.50
Mature fruit netting	.41	.71	.78	[1] 2.59	.42	1.13	[2] 2.94	1.24	.45	.25	.96	.90	.66

[1] Exceeds 0.05 point with *n* of 149. Wallace and Snedecor (*6*).
[2] Exceeds 0.01 point with *n* of 149. Wallace and Snedecor (*6*).

In the data presented in table 5, it can be seen that there is probably a significant difference between the determinate and indeterminate plants in all the characters presented with the exception of days to first male flower. Determinate growth habit is associated with short plant height, small number of mature fruit, small yield of mature

fruit, small weight per mature fruit, small total number of fruit (mature + green), small total weight of fruit (mature + green), small weight per fruit (mature + green), greater number of days to first female flower, short laterals, few laterals, and small total lateral length. The association between determinate growth habit and the characters pertaining to plant size may be the result of physiological causes. In other words, owing to the dwarfing effect of the determinate character, it may be impossible for determinate plants to develop the parts discussed to as great an extent as similar plants of indeterminate growth habit. Lateness of production of female flowers and small size of individual fruits may not be related to size of plant.

It is interesting to note, also, the association that is indicated in table 5 between average size per fruit, and spine color, spine size, spine frequency, fruit netting, and cream fruit color. Large fruit size appears to be associated with white spines, coarse spines, few spines, no netting, and cream fruit. As previously shown, white spines appear to be associated with cream fruit and no netting and cream fruit may be associated with no netting. If such is the case, one might expect that weight or size of fruit, when associated with one of these characters, would also be associated with the others, and, if the factors causing the expression of these characters are linked, at least one factor for size or weight of fruit would be present in the same linkage group. Furthermore, coarse spines appear to be associated with few spines but neither is associated with white spines or fruit netting and probably not with fruit color. This might indicate that the associated factors for coarse spines and few spines are in a different linkage group from the factors for spine color, fruit color, and fruit netting and that fruit size or weight appears to be associated with these characters, as well as with the group of associated characters mentioned above.

In table 5, the data also indicate a significant association between number of female flowers and orange fruit color; between number of laterals and spine size and frequency; and between total length of all laterals and red fruit color. Plants with orange-colored fruit had a smaller number of female flowers than red- yellow- or cream-fruited plants; plants with coarse, few spines had more laterals than plants with fine numerous spines; and plants with red fruit color had a greater total length of laterals than orange- or yellow-fruited plants. However, the average total lateral length per cream-fruited plant was slightly larger than that of the red-fruited plants, but owing to the fact that there were only 10 plants in this group as compared to 82 in the red-fruited group, the standard error was so high that the mean of the cream-fruited group did not differ significantly from the mean of the experiment.

INDEPENDENT ASSORTMENT OF QUANTITATIVE CHARACTERS

In a further analysis of the data obtained in this experiment, correlation studies were made in all possible combinations between characters given in table 5, on which no study of the segregation was made Observations on149 to 156 individuals were used to measure the association. Table 6 is a presentation of the correlation coefficients obtained in this analysis.

TABLE 6.—*Correlation coefficients obtained between several plant characters in the F^2 progeny of a cucumber cross*

Characters correlated	Number of mature fruits	Total weight of mature fruit	Weight (size) per mature fruit	Total number of fruits (mature+green)	Total weight of fruit (mature+green)	Weight per fruit (mature+green)	Days to first male flower	Days to first female flower	Number of female flowers	Number of laterals	Length per lateral	Total length of laterals
Plant height (inches)	[1] 0.74	[1] 0.82	[1] 0.51	[1] 0.81	[1] 0.86	[1] 0.49	0.10	[2] −0.19	[1] 0.78	[1] 0.67	[1] 0.48	[1] 0.76
Number of mature fruits	.....	[1] .91	[1] .24	[1] .86	[1] .83	[1] .34	.11	[1] −.22	[1] .78	[1] .53	[1] .54	[1] .72
Total weight of mature fruit	.....	.....	[1] .56	[1] .86	[1] .96	[1] .61	.14	[2] −.20	[1] .81	[1] .57	[1] .58	[1] .79
Weight per mature fruit	.....	.....	.....	[1] .39	[1] .63	[1] .90	.12	−.04	[1] .41	[1] .28	[1] .36	[1] .41
Total number of fruits (mature+green)	.....	.....	.....	.....	[1] .91	[1] .29	.13	[1] −.31	[1] .85	[1] .54	[1] .61	[1] .77
Total weight of fruit mature+green)	.....	.....	.....	.....	.....	[1] .60	.14	[1] −.24	[1] .84	[1] .57	[1] .61	[1] .80
Weight per fruit (mature+green)	.....	.....	.....	.....	.....	.....	.07	.02	[1] .37	[1] .28	[1] .29	[1] .36
Days to first male flower	.....	.....	.....	.....	.....	.....	.....	−.05	.15	.03	.10	.03
Days to first female flower	.....	.....	.....	.....	.....	.....	.....	.....	[1] −.27	−.01	[1] −.32	[1] −.20
Number of female flowers	.....	.....	.....	.....	.....	.....	.....	.....	.....	[1] .55	[1] .51	[1] .74
Number of laterals	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.09	[1] .67
Average length per lateral	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	[1] .73

[1] Exceeds 0.01 point with n of 149. Wallace and Snedecor (*6*).
[2] Exceeds 0.05 point with n of 149. Wallace and Snedecor (*6*).

The data presented in table 6 indicate a high degree of association between many of the characters studied. Most of these characters may be considered to be expressions of vigor of the plant, and it is interesting to note that the taller the plant is the larger are the other size or vigor characters. It will be noted also that days to the first female flower shows a significant negative correlation with all these characters, with the exception of average weight per fruit, number of laterals, and days to the first male flower.

TABLE 7.—*Correlation coefficients* [1] *obtained between the time of production of the first male and female flower and the time of fruit maturity in 2,195 F_3 plants of a cucumber cross*

Characters	Days after planting to first female flower	Days after planting to first female flower set	Days after planting to first mature fruit
Days after planting to—			
First male flower	0.0877	0.3770	0.2350
First female flower	.....	.7504	.6719
First female flower set	.....	.....	.8440

[1] All highly significant (*6*).

There appears to be no association between number of laterals and length of lateral. Neither does days to the first male flower appear to be significantly associated with any of the other characters studied. While there appears to be a negative, although not significant, asso-

ciation between days to first male flower and days to first female flower, studies conducted on the F_3 lines in 1937 to determine the relation of time of male and female flowering to fruit maturity indicate that the relationship should be positive (table 7). The failure to obtain a significant correlation in the F_2 study may have been due to the fact that there was only a difference of about 4 days between the mean time of flowering in the parents and that the number of individuals in the F_2 population was too small to bring out the real relationship.

RELATION OF TIME OF MALE AND FEMALE FLOWERING TO TIME OF FRUIT MATURITY

A study was made with 2,195 plants from F_3 lines to determine whether time of fruit maturity, as indicated by the initiation of the change from green to the mature fruit color, could be predicted from the time of production of the first male or female flowers. The results obtained in this study are presented in table 7.

The data given in table 7 indicate a highly significant association between time of first flower production and time of fruit maturity. However, time of production of the first female flower appears more closely associated with fruit maturity than time of production of the first male flower and probably provides a better basis for prediction of earliness of maturity. While the coefficient, 0.844, indicates that the first female flower to set is the best basis for predicting earliness of maturity, it is interesting to note that there is a fairly high correlation, 0.6719, between time of production of the first female flower and earliness of maturity. This may indicate that plant selection for earliness might be made on the basis of the first female flower to appear on the plant regardless of whether it develops into a fruit or not.

SUMMARY

A study was made of inheritance in a cucumber cross between parents differing from each other in height, growth habit, spine color, spine size, spine frequency, nature fruit color, and mature fruit netting. A study was also made of the association of these and a number of other characters related to growth, flowering, and yield. The results of these studies are presented in the following outline:

1. The inheritance of the following differential characters could be explained by assuming a single factor difference.
   - *a*. Determinate growth habit versus indeterminate.
   - *b*. Black spines versus white.
   - *c*. Coarse spines versus fine.
   - *d*. Few spines versus numerous.
   - *e*. Mature fruit netting versus slight or no netting.
   - *f*. Tall plant versus short or dwarf.
2. The inheritance of mature fruit color could be explained by assuming the interaction of two independently inherited factors, the ratio obtained being approximately 9 red: 3 orange: 3 yellow: 1 cream.
3. The factor responsible for determinate growth also tends to decrease the height of the plant.

4. Tests for independent assortment indicate that—
   a. The following characters appear to be associated with each other either by complete linkage or as an expression of the same factor.
      1. Coarse spines with few spines; fine spines with numerous spines.
      2. Black spines with red and orange fruit; white spines with yellow and cream.
      3. Black spines with heavy netting; white spines with no netting.
      4. Heavy netting with red and orange fruit; no netting with yellow and cream.
   b. Determinate growth habit appears to be associated with short plant, small number of mature fruit, small yield of mature fruit, small weight per mature fruit, small total number of fruit (mature+green), small total weight of fruit (mature+green), small weight per fruit (mature+green), greater number of days to first female flower, short laterals, few laterals, and small total lateral length.
   c. Large fruit size (weight) appears to be associated with white spines, coarse spines, few spines, no netting, and cream fruit.
   d. The data indicate that more than one factor may be involved in the expression of fruit size or weight.
   e. Number of female flowers appeared to be associated with fruit color; plants with orange fruit having fewer female flowers than those with red, yellow, or cream.
   f. Number of laterals appeared to be associated with spine size and frequency; plants with coarse, few spines having more laterals than plants with fine, numerous spines.
   g. In correlation studies in the F_2 progeny, plant height, number of mature fruits, total weight of mature fruit, weight (size) per mature fruit, total number of fruits (mature+green), total weight of fruit (mature+green), average weight per fruit (mature+green), number of female flowers, number of laterals, average length of lateral, and total length of laterals showed a positive and, in most cases, a highly significant association with each other when correlated in all possible combinations. Days to production of the first female flower showed a negative and, in most cases, a significant association with all the above characters. Days to the first male flower showed a positive but not significant association with the above characters.
5. A high degree of association was found between the time of the production of the first female flower by the plant and maturity of the fruit.

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FRENCHING OF TOBACCO DISTINGUISHED FROM THALLIUM TOXICITY BY SPECTROGRAPHIC ANALYSIS [1]

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INTRODUCTION

Several theories as to the cause of tobacco frenching have been proposed, but each in its turn has been found to be erroneous. Recently, it has been postulated that frenching is probably a symptom of thallium toxicity. Since the demonstration of the presence of thallium in frenched tobacco tissue in toxic amounts or its absence is essential to an evaluation of the thallium-toxicity theory, and the quantity of thallium necessary to produce thallium toxicity under experimental conditions is too minute to be detected by ordinary chemical methods, this theory at first seemed impossible to prove or disprove. However, since spectrographic analysis has been shown by a number of investigators (*9, 10, 16*)[2] to be applicable to the determination of minute quantities of minerals in plant tissues, the spectrographic method seemed to be applicable to the evaluation of the thallium-toxicity theory. Accordingly, a cooperative study was undertaken in 1937 between the Virginia Agricultural Experiment Station and the Physics Department of the Virginia Polytechnic Institute to determine by spectrographic analysis whether there is any relationship between frenching and thallium toxicity of tobacco. Preliminary reports on this study have been made by the authors.[3]

REVIEW OF LITERATURE

Results reported by Shear (*11*) in 1933 led him to propose a toxicity theory to explain the cause of frenching, although the nature of the toxin was not determined. Karraker and Bortner (*6*), although they state that they do not think frenching is of toxic origin, present no evidence that is incompatible with such a theory, and the following quotations from the summary of their work, published in 1934, are most easily explainable by such a theory (*6, p. 108*).

Frenching in plants in soil and river sand in the greenhouse was rather easily brought about by proper addition of pulverized limestone and adjustment of

[1] Received for publication October 2, 1939. Paper No. 106 of the Section of Botany and Plant Pathology, Virginia Agricultural Experiment Station. This paper was awarded the Jefferson medal of the Virginia Academy of Science at the seventeenth annual meeting, May 5, 1939, Danville, Va. This project was partly financed by a research grant from the Virginia Academy of Science.

[2] Italic numbers in parentheses refer to Literature Cited, p. 139.

[3] SHEAR, G. M., and USSERY, H. D. SPECTROGRAPHIC ANALYSIS APPLIED TO THE STUDY OF TOBACCO FRENCHING. Paper presented at 15th annual meeting Amer. Soc. Plant Physiologists. Title in Abstracts and Titles of Papers, p. 19. 1938. [Mimeographed.]

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nutrient supply. It was more difficult to obtain frenching when acid-treated and washed white silica sand was used.

* * * * * * *

While the results of the studies rather clearly show frenching to be related to soil reaction and nutrient supply as described, yet for various reasons it can not be concluded that other causal factors are not present with which or thru which the reaction and nutrient factors may function. And it may be, even, that reaction and nutrient factors merely suppress or bring into activity some other factor which is the underlying cause, somewhat as moisture supply affects frenching by affecting rate of growth.

The treatment of the sand referred to above should not add anything to the sand but should tend to remove most soluble material contained in it. If, thereby, the tendency toward frenching is reduced the washing must have removed something from the sand which was toxic to the tobacco. If, as the authors suggest, the combination of soil reaction and nutrient supply may bring into activity some other factor which is the underlying cause, then that factor must be a toxic substance of some kind.

In 1935 Spencer (*12*) reached the conclusion that frenching was not a mineral-deficiency disease but was a toxicity disease. At that time he proposed no explanation as to the cause of the toxic effect.

Thallium toxicity was first compared to frenching of tobacco by McMurtrey in 1932 (*8*). He says:

Whether the typical frenching disease of tobacco is due to thallium toxicity cannot be definitely stated at this time, but it appears that there is much in common in growth manifestations exhibited by the two pathological conditions.

Spencer (*13*) made an extensive study of frenching and thallium toxicity and showed that in most respects the symptoms of the two are the same. He found that different species of tobacco each showed the same susceptibility or resistance to both frenching and thallium toxicity, and that both disorders were completely controlled by applications of available nitrogen, aluminum, or potassium iodide, and partly controlled by steam sterilization. He found that smaller applications of thallium salts were required to produce a toxic effect on tobacco grown on soil that caused frenching than on nonfrenching soils. He concludes his paper with the following statement:

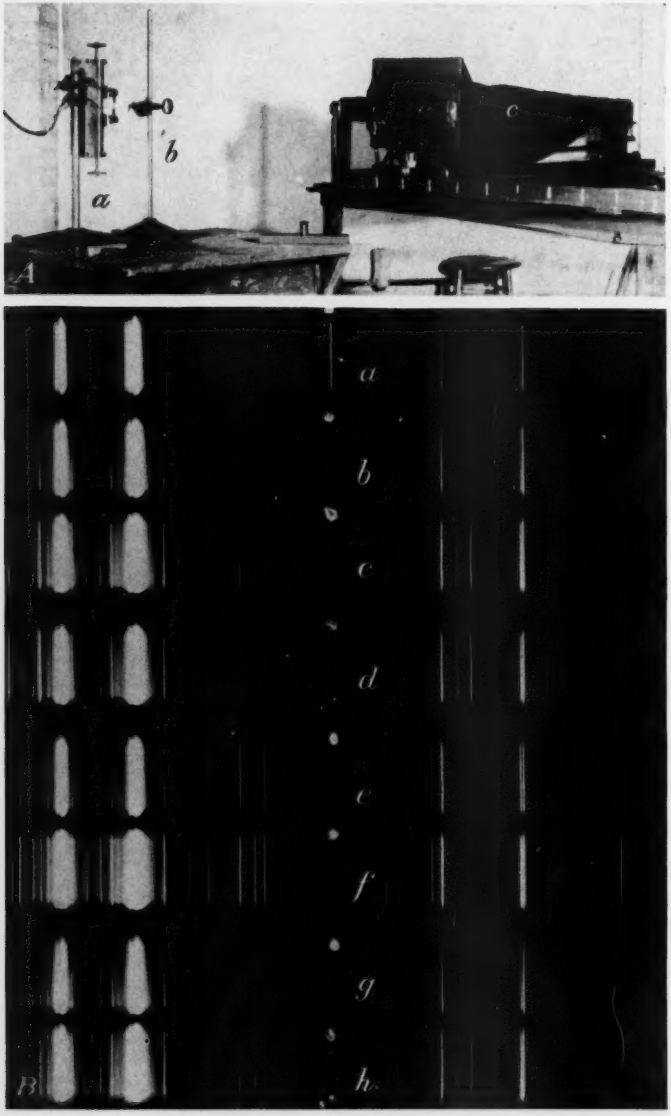
The experimental evidence reported indicates a striking similarity between natural frenching and thallium toxicity not only with respect to the symptoms they produce on Turkish tobacco but also with regard to the methods by which they are controlled. Final proof that these two diseases are identical requires the demonstration of the presence of toxic quantities of thallium in soils which produce frenching. At present it is questionable whether the known chemical methods are sufficiently sensitive to detect such small traces of thallium as the experiments here reported have indicated must be present in the soil if frenching is a thallium-toxicity disease.

The following statement appearing in Connecticut Experiment Station Bulletin 410 (*1, p. 406*), further emphasizes the difficult nature of this problem:

The real difficulty, however, in identifying the two maladies, lies in the fact that there are no known methods of measuring and comparing the amounts of thallium contained in frenched tobacco leaves and in those known to be injured by thallium.

After the presentation of the paper by Shear and Ussery[4] in December 1938, in which they presented positive spectroscopic evidence to

[4] See footnote 3.



A.—Equipment used in making spectrograms. *a*, Arc stand; *b*, quartz lens; and *c*, grating spectrograph.

B.—Spectrograms of tobacco ash showing region of spectrum where the 3775.73 A thallium line occurs. The dot above each spectrogram indicates the wavelength at which the thallium line should appear. *a*, Ash to which a drop of dilute thallous nitrate solution was added; *b*, ash of leaves showing severe chlorosis due to thallium toxicity; *c*, ash of leaves showing mild chlorosis; *d*, ash of leaves from plants grown in culture solution containing thallium but showing no chlorotic symptoms; *e* and *f*, soil from eastern and western Virginia on which frenching of tobacco occurred; *g* and *h*, ash of frenched leaves from peat and sand culture and from frenched plants grown in the field.

show that the two disorders are not the same, Spencer and Lavin (*14*) published a brief article based on very limited spectroscopic data in which they concede that frenching and thallium toxicity may not be the same.

METHODS OF EXPERIMENTATION

The tobacco used in this study consisted of varieties of *Nicotiana tabacum* L.

The plants receiving thallium as thallium nitrate were grown in water culture. Six or eight plants were grown in each treatment, and representative material from each plant in a treatment was collected for each analysis. The standard nutrient solution used made available the following amounts of elements per plant:

Element:	*Milligrams*
Nitrogen	406
Phosphorus	56
Potassium	487
Calcium	316
Magnesium	40
Chlorine	48
Sulfur	40
Sodium	30
Manganese	1.2
Iron	.99
Boron	.85

For the lower concentrations of nutrients, dilutions of this formula were used.

The plant tissue to be analyzed was dried in an oven and then ashed at a dull red heat in porcelain crucibles over a gas burner. Twenty-four milligrams (±0.5 mg.) of ash was packed into the hollowed end of the lower + electrode for analysis.

The electrodes were made from No. 3 B. & S. gage copper wire. The ends of the electrodes were cut down on a lathe to 4 mm. in diameter. The diameter of the hole in the lower electrodes was 3.5 mm.

A grating spectrograph[5] with a dispersion of 9.43 A. per millimeter in the first order and a resolving power of 25,000 was used in making this study. The spectrograph and arc stand are shown in plate 1, *A*. The arc was operated by a 120-volt direct current generator, the current used varying from 8 to 10 a. The arc was focused on the slit by means of a quartz lens. An exposure of 30 seconds was used for all the plant ash samples herein reported.

As a densitometer was not available for making quantitative determinations a direct visual comparison was made by the method of homologous pairs which Gerlach and Schweitzer (*2*) used in evaluating spark spectra. Various amounts of thallium were added to samples of tobacco ash that showed no thallium. Seventy-two milligrams of such ash was wet with enough thallium nitrate solution to give the desired percentages of thallium. After being thoroughly dried and mixed, 24-mg. samples were used in making the determinations. On each spectrogram of an ash containing a known amount of thallium a copper line of equal density and as near the thallium line as possible was selected and used as a basis for evaluating the spectrograms of the ash samples.

[5] Grating ruled by Dr. J. A. Anderson.

When the density of the line at 3,779.3 A. matched that of the 3,775.7–A. Tl line the ash contained no thallium but the exposure had been long enough to cause the thallium in the copper to appear. If the 3,775.7–A. Tl line matched the 3,780.0–A. Cu line then the ash contained less than 0.0001 percent of thallium. When the 3,775.7–A. Tl line matched the 3,800.4–A. Cu line the ash contained 0.0002 percent of thallium. When the 3,775.7–A. Tl line matched the 3,759.5–A. Cu line the ash contained 0.0004 percent of thallium. When the 3,775.7–A. Tl line matched the 3,771.9–A. Cu line the ash contained 0.0006 percent of thallium. When the 3,775.7–A. Tl line matched the 3,741.2–A. Cu line the ash contained 0.001 percent of thallium. When the 3,519.2–A. Tl line matched the 3,520.0–A. Cu line the ash contained 0.005 percent of thallium. In these spectrograms, the criterion for proper exposure was that the pair of copper lines 3,450.33 A. and 3,457.85 A. were a visual match in density, since these lines were found to vary in relative intensity with time of exposure and conditions of excitation. The wave-length values used were those listed by Kayser (7).

EXPERIMENTAL DATA

During the course of this study, approximately 200 spectrograms were made, about half of which were of ash samples. The original plates were used exclusively in determining the amount, if any, of thallium present in the ash samples analyzed.

The first step in this study of the tobacco ash samples was to determine whether thallium could be detected in the ash of healthy and frenched tobacco grown on different soils. Six samples of frenched leaves were analyzed. Four of these were from field-grown plants collected in southwest, central, and eastern Virginia, and from central North Carolina. Two of the frenched samples came from plants grown in the greenhouse, one in soil and one in a peat-sand mixture. Healthy leaves from field-grown plants, from plants grown in frenching soil that had been steamed, and from plants grown in culture solutions receiving no thallium were used. Two soils which caused frenching of tobacco were also analyzed. None of these samples showed any indication of thallium in the spectrograms. Several representative spectrograms are shown in plate 1, B.

To determine whether or not thallium could be detected in the ash of plants which had received small amounts of this substance, tobacco plants were grown in nutrient solution to which thallium nitrate was added. Two sets of eight plants each were grown in full strength nutrient solution for 1 week and then thallium was added at the rate of 0.10 p. p. m. to one set and 0.05 p. p. m. to the other set. The nutrient solution was not renewed but additional thallium was added at weekly intervals for 3 weeks, when chlorosis due to thallium toxicity appeared on some of the plants. At this time leaf samples were collected for analysis. Three samples from the plants receiving thallium were taken, one of the leaves showing severe chlorosis, one of the leaves showing mild chlorosis, and one of the leaves showing no chlorosis. On analysis the ash of these samples gave 0.0006, 0.0003,

and 0.0002 percent of thallium respectively, thus showing a relationship between the severity of the symptoms and the amount of thallium in the tissue.

Spencer, in discussing these results with the senior author on December 29, 1938, stated that the results might not be conclusive in showing the two disorders to be distinct since only one concentration of nutrient solution was used. His results had indicated that the amount of thallium required to cause toxic symptoms decreased with a decrease in nutrient content of the solution in which the plants were grown; hence, there was a possibility that the nutrient solution could be diluted to a point where the thallium required to produce toxic symptoms could not be detected in the leaf ash by spectrographic analysis.

A series of experiments was therefore conducted in which the nutrient solution and the amount of thallium added were varied to determine their effect on the appearance of chlorosis and the percentage of thallium in the ash. The results obtained from the first sets of plants are shown in tables 1 and 2. Plants with stems 1 to 2 inches long were grown 1 week in standard-strength nutrient solution. Twelve plants were then placed in each of the following solutions: Standard strength, two third standard strength, and one third standard strength. Half of the plants in each of these groups received 0.067 p. p. m. of thallium, while the other half received 0.033 p. p. m. No further treatment was given these plants except for aerating the solution twice daily and maintaining the solution at a constant level by adding distilled water. The plants were growing rapidly when the thallium was added and, with the exception of those in the weakest nutrient solution, there was no noticeable difference in growth or appearance of plants. The plants in the one third strength nutrient solution did not grow so rapidly as the others and after about 2 weeks the young leaves developed a diffuse chlorosis not typical of the chlorosis produced by thallium.

TABLE 1.—*Thallium in tobacco ash from young leaves of tobacco grown in culture solutions of varying nutrient and thallium content; samples taken 1 and 2 weeks after addition of thallium*

Concentration of nutrient solution	1 week after addition of thallium		2 weeks after addition of thallium	
	Concentration of thallium in nutrient solution	Concentration of thallium in leaf ash	Concentration of thallium in nutrient solution	Concentration of thallium in leaf ash
	P. p. m.	*Percent*	*P. p. m.*	*Percent*
Standard	0.067	0.0001	0.067	0.0001
Do	.033	(1)	.033	(1)
Two-thirds standard	.067	(1)	.067	.0001
Do	.033	(1)	.033	(1)
One-third standard	.067	(1)	.067	.0006
Do	.033	(2)	.033	.0003
From soil			(3)	(3)

[1] Present but less than 0.0001 percent.
[2] Presence doubtful.
[3] None.

TABLE 2.—*Thallium in tobacco ash from different parts of tobacco plants grown in one-third standard strength culture solutions of varying thallium content; samples taken 3 weeks after addition of thallium*

Portion of plant used	Concentration of thallium in nutrient solution	Concentration of thallium in ash of root, stem, or leaf	Portion of plant used	Concentration of thallium in nutrient solution	Concentration of thallium in ash of root, stem, or leaf
	P. p. m.	*Percent*		*P. p. m.*	*Percent*
Roots	0.067	0.005	Lower leaves	0.067	0.001
Do	.033	.002	Do	.033	.0002
Stems	.067	.0003	Upper leaves	.067	.0001
Do	.033	.0002	Do	.033	[1]

[1] Present but less than 0.0001 percent.

Samples consisting of young leaves from each treatment were collected 1 week and 2 weeks after the thallium was added. Each sample consisted of leaves from all the plants receiving the same treatment. The first set of samples showed the greatest amount of thallium in the leaves of plants grown in standard strength nutrient solution plus 0.067 p.p.m. of thallium. The presence of thallium was doubtful in the sample from plants in one-third standard strength nutrient solution plus 0.033 p. p. m. of thallium. All the samples between these extremes showed essentially the same amount of thallium. By the end of the second week this relationship had changed very markedly. The plants receiving the larger amount of thallium showed a higher percentage of thallium than the plants receiving the same strength of nutrient solution but a smaller amount of thallium. The thallium in the ash of plants in the one-third strength nutrient solution receiving 0.067 p. p. m. and in the one-third strength nutrient solution receiving 0.033 p. p. m. of thallium had reached 0.0006 and 0.0003 p. p. m., respectively.

After the plants had grown for 3 weeks in the thallium solutions the experiment was discontinued as the plants were becoming crowded. The two sets of plants in the one-third strength nutrient solution were separated for analysis into root, stem, old leaf, and young leaf tissue. The results of these analyses are shown in table 2. It may be seen from these results that the greatest amount of thallium is found in the roots, with an intermediate amount in the stems and older leaves and the smallest amount in the young leaves. In each case the ash of plants receiving the least thallium gave a smaller percentage of this material than was found in the ash of the same part of the other plants.

When the next series of plants was harvested, the six plants in the one-third strength nutrient solution receiving 0.033 p. p. m. of thallium were divided for analysis into lower half of root system, upper half of root system, lower part of stem, old leaves, and young leaves. Although these plants had been in the nutrient solution twice as long as the previous set the plants were about the same size because they were grown in distilled water for several days before the nutrient solution was added, which slowed down their growth rate.

The results of these analyses were as follows:

Part of plant used: [1]	*Concentration of thallium in ash, percent*
Lower half of root system	0.0002
Upper half of root system	Between 0.0006 and 0.0008
Stems	Present but less than 0.0001; more than in old leaves
Old leaves	Present but less than 0.0001; more than in young leaves
Young leaves	Present but less than 0.0001

[1] Samples taken 6 weeks after addition of thallium.

These results substantiate those of the previous analyses in that they show a marked gradient in thallium decreasing from the roots to the youngest leaves.

It will be noted that the percentage of thallium in the ash of the young leaves had significantly decreased from the second to the third week after treatment. Since no marked toxic effects were observed in the first sets of dilutions of nutrient solution, other sets were prepared in which still more dilute solutions with different amounts of thallium were used. In order to reduce the nutrient reserve in these plants they were grown in distilled water for 4 days before nutrients and thallium were added. This experiment included groups of plants receiving one-third strength, one-sixth strength, and one-twelfth strength nutrient solution, half of the plants in each group receiving in addition 0.067 p. p. m. of thallium and the other half 0.033 p. p. m. The results of this experiment are shown in table 3. The plants in these groups grew very slowly and all showed a marked toxic effect on their roots. The roots stopped growing as soon as they penetrated the solution and a mat of short roots formed just above the surface of the solution. After 2 weeks the roots of the plants in the one-third strength nutrient solution with 0.033 p. p. m. of thallium were able to grow in the solution without further injury, and the severity of the root injury was reduced somewhat in the one-third strength nutrient solution with the larger amount of thallium. The roots of the other plants showed no indications of recovery after 4 weeks in the solutions. Samples were collected from these plants 2½ weeks and 4 weeks after treatment. The spectrograms of the first samples could not be used as the equipment was not adjusted properly; and since there was originally only a small amount of material available, there was not sufficient ash to repeat the analyses on this set of samples. The results shown are from the second set of samples. All of these samples showed measurable amounts of thallium but the differences between the samples were not great.

TABLE 3.—*Thallium in tobacco ash from young leaves of tobacco grown in culture solutions of different nutrient and thallium content*

Concentration of nutrient solution	Concentration of thallium in nutrient solution	Concentration of thallium in ash	Concentration of nutrient solution	Concentration of thallium in nutrient solution	Concentration of thallium in ash
	P. p. m.	*Percent*		*P. p. m.*	*Percent*
One-third standard	[1] 0.067	0.0003	One-twelfth standard	[1] 0.333	0.0002
Do	[1] .033	.0003	Do	[2] .016	[3]
One-sixth standard	[1] .067	.0002	Do	[2] .008	[4]
Do	[1] .033	.0002—	Do	[2] .000	[4]
One-twelfth standard	[1] .067	.0003			

[1] Samples collected 4 weeks after addition of nutrients and thallium.
[2] Samples collected 1 week after addition of nutrients and thallium.
[3] Present but less than 0.0001 percent.
[4] None.

After the plants had grown in the thallium solutions for 3 weeks, typical thallium-induced chlorosis appeared on leaves of plants in three different treatments. Five plants out of six in the one-sixth strength nutrient solution plus 0.067 p. p. m. of thallium showed mild chlorosis, but by the end of the fourth week the youngest leaves appeared healthy. Three plants out of six in the one-twelfth strength nutrient solution plus 0.067 p. p. m. of thallium developed chlorosis which showed up on the youngest leaves at the end of 5 weeks. Two plants in the one-twelfth strength nutrient solution plus 0.033 p. p. m. of thallium developed a mild chlorosis which no longer appeared on the youngest leaves after 4 weeks.

With another group of plants in one-twelfth strength nutrient solution the thallium content was reduced to 0.016 and 0.008 p. p. m. (table 3), and after 1 week a trace of thallium could be detected in the leaves of plants receiving 0.016 p. p. m. of thallium but none could be detected in the plants receiving the smaller amount.

DISCUSSION

It might be argued that by further manipulation of the nutrient solution, such as an alteration in the ratio of the various nutrients, leaf chlorosis might be produced with a smaller amount of thallium available to the plant, but such an assumption would not seem plausible on the basis of the experiments reported here. The ratio of the nutrients used in the nutrient solutions employed in these studies was based on a number of analyses of healthy field-grown tobacco and therefore the ratio of the various elements should be nearly optimum. It would, therefore, appear that no one element could be significantly reduced without a corresponding reduction in growth. The nutrient level was reduced in this study until very little growth was produced, and yet thallium in too small an amount to cause chlorosis could be detected. Since in most cases of frenching growth is more rapid than this, thallium, if the causal factor, would be present in measureable quantities. In this study the smallest amount of thallium added to the nutrient solution that could be detected in the plant ash was only one-fourth the minimal amount required in culture solution to cause chlorosis, according to Spencer (*13*).

The results show a gradient in thallium in the tobacco plant, decreasing from the roots to the tip of the shoot. This gradient existing several weeks after the addition of the thallium indicates that it is deposited in an immobile state in the plant. It might be precipitated in an organic or an inorganic compound. Since the thallium is not precipitated in the nutrient solution its precipitation in the plant as an inorganic salt would have to be brought about by some chemical change effected by the metabolism of the plant. Whatever the compound in which the thallium is fixed, its components or catalyzer must be present in extremely minute quantity; otherwise with the small amount of thallium available all of it should be precipitated in the roots. The fact that a gradient occurs would support such an hypothesis.

Since the results of these experiments are so conclusive in showing that frenching is not thallium toxicity, it might be well to point out facts from the results of other investigators that could not be fitted into the frenching-thallium toxicity theory. In the first place

the symptoms of the two disorders are not identical, as was pointed out by McMurtrey (*8*). In the case of thallium toxicity, the first indications of chlorosis are along the midrib or the leaf; whereas in frenching, chlorosis starts at the borders of the leaf. Spencer (*12*) showed that 1 part of frenching soil in 2,000 parts of washed quartz sand was sufficient to cause frenching. He has also shown that it takes approximately 0.10 p. p. m. of thallium in sand to cause thallium-toxicity symptoms to appear on tobacco grown in the sand. If these two disorders were the same, then the frenching soil would contain about 400 pounds of soluble thallium per acre or 200 p. p. m., which is 800 times as much as was required to produce thallium toxicity symptoms on tobacco in one of the soils with which he worked. His finding, that less thallium was required to produce toxic symptoms on tobacco growing in frenching soil than in other soil, does not necessarily mean that the thallium which he added supplements some already in the frenching soil; it might just as readily mean that the absorptive power of such a soil was low and therefore neither the thallium nor the frenching toxin was fixed as they might be in a soil that would not produce frenching.

Steinberg (*15, p. 574*), in a recent paper on the essentiality of gallium to growth and reproduction of *Aspergillus niger*, makes the following statement:

> The reported toxicity of thallium, a chemical homologue of gallium, to tobacco by McMurtrey, in association with the close duplication of the symptoms of frenching, would appear to indicate the possibility that thallium prevented the utilization of gallium by the plant.

He draws the analogy on the basis of Hurd-Karrer's work in which she shows that the toxic effects of selenium, arsenic, rubidium, and strontium can be prevented by sulfur, phosphorus, potassium, and calcium respectively. She concludes from these findings that the salt of a toxic element can be rendered nontoxic to plants through the addition of a sufficient amount of the salt of an essential element in the same chemical group. She clearly shows that the toxic action is not due to the injurious element preventing the absorption or utilization of the essential element, which is just the reverse of the assumption upon which Steinberg appears to base his hypothesis. It is entirely possible on the basis of Hurd-Karrer's results (*3, 4, 5*) that gallium might, if present in sufficient amount, counteract thallium toxicity, although its absence could not be construed as the cause of the symptoms produced by thallium.

An examination of the spectrograms in the region of the persistent gallium lines at 4,033.01 and 4,172.05 A. revealed no indications of gallium in any of the ash samples. The chemical properties of gallium are such that it would be more easily excited in the arc than thallium and therefore should be detectable in more minute amounts than thallium. Thus if gallium is essential for the growth of tobacco the amount necessary must be less than 0.0001 percent in the ash.

The theory that frenching is caused by an unstable organic toxin which accumulates only under certain abnormal conditions and which is produced by some widely distributed soil organism is herewith proposed. This theory is based on a number of facts which have been discovered concerning the conditions under which frenching develops. Frenching has been reported from most regions in the

world where tobacco has been grown, proving the wide distribution of the underlying cause. Toxic minerals which might conceivably cause frenching are not so universally distributed, whereas certain types of organisms are. Partial sterilization of the soil temporarily renders it incapable of producing frenching. The instability of the toxic factor has been shown by several experiments. A frenched plant recovers rapidly when the ball of soil in which it is growing is tapped from the pot and lightly replaced. Frenching could be produced in sand cultures watered with the leachate of frenching soil when the leachate was conducted from the soil to the sand through a separatory funnel. When the soil and water were mixed and the supernatant liquid used for watering sand cultures no frenching developed.

Frenching cannot be produced readily on tobacco in water cultures. Under such conditions very few normal soil organisms can grow. Frenching has been produced on tobacco in sand cultures under various conditions. Spencer, for example, obtained frenching on tobacco seedlings growing in sand containing 1 part of frenching soil in 2,000 parts of sand, a result which supports the organic-toxin theory, since the influence of such a small amount of soil could be explained most logically on the basis of its being an inoculum of soil organisms. He found that frenching could be produced in plain sand if tobacco were grown in the sand for a sufficient time. This might be explained on the basis of there being insufficient organic matter in the sand until some dead tobacco roots accumulated.

SUMMARY AND CONCLUSIONS

A spectrographic analysis of the ash of tobacco samples from healthy, frenched, and thallium-treated plants was made. Samples of soil in which frenched tobacco had occurred were also analyzed.

Thallium could be detected only in samples of ash from plants grown in a nutrient solution to which thallium had been added, proving that frenching and thallium toxicity are not the same. Thallium could be detected in ash samples in amounts of less than 0.0001 percent and in ash samples from plants receiving only 0.016 of a part per million of thallium in the nutrient solution.

The weaker the nutrient solution the smaller the amount of thallium necessary to produce toxic symptoms.

Thallium is fixed in the plant tissue in such a way as to produce a gradient, decreasing from the roots to the youngest leaves. The plant, therefore, must have a continuous supply of available thallium if thallium toxicity symptoms are to continue to appear on the new growth as it is produced.

Gallium does not appear to have any relationship to frenching or thallium toxicity.

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THE VITAMIN A ACTIVITY AND THE VITAMIN B_1 CONTENT OF SOYBEANS AND COWPEAS[1]

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INTRODUCTION

In view of the wide use of soybeans (*Soja max*) and cowpeas (*Vigna sinensis*) as food for both man and animals, and the relative scarcity of data concerning their vitamin A activity and vitamin B_1 content, it seems desirable to publish the results of assays made on several varieties of these leguminous seeds.

The available results of vitamin assays of these seeds have been compiled by Daniel and Munsell (*1*)[2] and by Fixen and Roscoe (*2*). They are summarized in table 1.

TABLE 1.—*Carotene and vitamin A and B_1 content of soybeans and cowpeas as reported by various workers*

Product	Carotene per gram	Vitamin A activity per gram	Vitamin B_1	
			Per gram	Per gram
	Micrograms	*Sherman units*	*Sherman units*	*International units*
Soybean (*Soja max*):				
Dried [1]			1.4	
Yellow [1]			7.7	
Mammoth Yellow [1]			4.0	
Soya bean (*Glycine soja*) [2]	4.5–9.7			1.0, 1.5
Yellow [3]				<0.45
Black [3]				1.0
Soybean [3]	0.4–0.8			
Mammoth yellow [4]	.50			
Tokio [4]	.49			
Tarheel black [4]	.38			
Biloxi [4]	.37			
Rokusun [4]	.29, .38			
36 other varieties [4]	.17–2.44			
Cowpea (*Vigna sinensis*) [3]				1.7
Dried [1]		2.0		
Black-eyed [1]		3.0		
Cream Crowder [4]	.33, .25			
Brown Crowder [4]	.26			
Virginia blackeye [4]	.27			
5 other varieties [4]	.26–.33			

[1] Data from Daniel and Munsell (*1*).
[2] Data from Fixen and Roscoe (*2*).
[3] Data from Lanzig and Van Veen (*3*).
[4] Data from Sherman and Salmon (*6*).

EXPERIMENTAL PROCEDURE

Vitamin B_1 was determined by the method described by Sherwood and Halverson (*7*).

The basal ration for vitamin A assays consisted of purified casein 18 percent, cornstarch 54 percent, irradiated dried yeast 7.5 percent, Wesson salt mixture (*8*) 3.5 percent, agar-agar 2 percent, and hydrogenated vegetable fat 15 percent.

[1] Received for publication October 16, 1939. Paper No. 109 of the Technical Series, North Carolina Agricultural Experiment Station.
[2] Italic numbers in parentheses refer to Literature Cited, p. 143.

The mothers of the rats used for the assays received Sherman's diet B as modified by Russell (*5*) until the litters were 12 to 14 days old, when they were changed to the above-described vitamin-A-free basal ration. After weaning, the young rats were kept on the basal ration until they ceased to gain in weight. Almost invariably at this time, about 35 days after weaning, ophthalmia was moderately severe. The rats were then placed in individual screen-bottomed cages, continued on the basal ration, and one-half of them were given daily 1 gm. of the sample being tested. The remaining animals were kept as negative controls.

The sample is reported as free from vitamin-A activity when the test rats, usually 4 for each sample continued to lose weight and died in about the same length of time as their controls. Those samples that promoted growth in the test rats and alleviated the ophthalmia were assayed quantitatively. Usually each of 10 rats was fed daily 1 gm. of the sample being assayed, and at the same time an equal number of rats were given daily definite doses (at least 2 planes) of the International Standard carotene dissolved in refined cottonseed oil (Wesson oil). All rats received the basal ration ad libitum. The standard carotene solution was sealed in glass ampoules under nitrogen gas as soon as made and kept in the freezing compartment of an electric refrigerator until needed. After an ampoule was opened its contents (enough for 3 to 7 days) were kept in the refrigerator when not in actual use.

One sample of Tokyo soybeans was grown on the experiment station farm and one of Rokusun soybeans was obtained through the courtesy of Charles Dearing from the Coastal Plain test farm. All others were obtained from a local seed dealer.

All samples were of high viability [3] and were carefully hand picked, only seed of uniform color and size being retained for the assays. The samples were kept in the refrigerator until assayed.

RESULTS OF ASSAYS

The results of the assays are given in table 2.

TABLE 2.—*Vitamin A and B_1 content of different varieties of soybeans and cowpea*

Variety	Vitamin A per gram	Vitamin B_1 per gram	Variety	Vitamin A per gram	Vitamin B_1 per gram
	International units	*International units*		*International units*	*International units*
Soybeans:			Cowpeas:		
Laredo	1.3	4.1	Whippoorwill	0	2.3
Herman	.5	3.6	Brabham	0	2.7
Tokyo	0	3.2	Groit	.5	3.0
Do	.5	3.2	Blackeye	0	3.5
Biloxi	0	3.9	Iron	0	3.5
Do	0	3.5	Clay	0	3.7
Mammoth Yellow	0	3.8	Sugar Crowder (cream)	0	2.6
Do	0	3.2	Sugar Crowder (brown)	.5	2.6
Otootan	1.3	3.7	Small Black	0	2.8
Do	.5	4.1	Do	0	3.1
Virginia	0	4.8			
Do	.5	4.3	Average		3.0
Tarheel Black	.5	3.3			
Do	0	4.1			
Rokusun	0	3.9			
Average		3.8			

[3] Tested through the courtesy of J. W. Woodside of the seed laboratory of the State Department of Agriculture.

The results of the biological tests for vitamin-A activity of soybeans are in accord with those of animal feeders, who find that soybeans "supply no appreciable amount of vitamin A" (*4, p. 370*).

The quantities of carotene in the named varieties of soybeans and cowpeas in table 1 are equivalent to 0.4 to 0.8 International Unit of vitamin A per gram. These figures do not differ significantly from those in table 2 when it is considered that the carotene assay is capable of evaluating much smaller quantities than the biological method and that the amounts present are at or below the lower limit of the latter. Some difference between the two sets of assays is to be expected because of seasonal and other variations within the varieties, because of differences in maturity when harvested (*9*), and because all of the carotene present may not be utilized by the animals (*9*).

The varieties of soybeans assayed contained somewhat more vitamin B_1 than the cowpeas, although the differences are not great, and some samples of cowpeas were as rich in this factor as some of the soybeans. The values in table 2 are two to four times as great as those previously reported (table 1). The data given in table 1, however, are too meager to warrant any detailed comparisons. Both soybeans and cowpeas may be regarded as excellent sources of vitamin B_1.

SUMMARY

Nine common varieties of soybeans and eight of cowpeas, 15 and 10 samples respectively, were purchased from a seed store and assayed by the rat-growth method for their vitamin-A activity and vitamin-B_1 content.

The results show that none of these varieties of soybeans or cowpeas contain appreciable quantities of vitamin A. The soybeans contain 3.2 to 4.8 International Units of vitamin B_1 per gram and the cowpeas 2.3 to 3.7. There are no marked differences between varieties.

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